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Evidences of the importance of pantothenic acid during reproduction in rats

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NOTE TO USERS

EVIDENCES OF THE IMPORTANCE OF PANTOTHENIC ACID DURING REPRODUCTION IN RATS

by

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TABLE OF CONTENTS

INTRODUCTION

Over a period of several years, workers in the Home Economics Section of the Iowa Agricultural Experiment Station have been interested in the requirements of the B-vitamins for normal reproduction in rats. This interest resulted from the observation that rats were unable to reproduce normally on a ration containing partially dried autoclaved pork muscle and yeast as the principal sources of the B-vitamins and protein (Swanson, Armstrong and Nelson, 1943; Armstrong and Swanson, 1943). Resorptions, development of a syndrome resembling that of toxemia of pregnancy and the production of non-viable young occurred among animals fed this ration. Since fresh beef liver as a supplement to the diet prevented the disorders, it was believed that a deficiency of one or more of the B-vitamins might be responsible for the poor reproductive performance of the animals.

Soon after these observations were made it became evident that the ingredients of the diet were varying in composition as new sources of supplies were purchased. In recent years the occurrence of toxemia has been sporadic, and the number of resorptions and the viability of the young has varied.

In an attempt to discover which factors were present in suboptimal amounts in the pork diet, the concentrations of thiamine, riboflavin, niacin, biotin, and pantothenic acid were measured in the diets and in the tissues of pregnant animals on the day of parturition (Everson, Williams, Wheeler, Swanson, Spivey and Eppright, 1948; Williams, 1947). In a comparison of the pork diet with a modified Steenbock ration which has been used successfully in the stock colony of this laboratory over a period of years, it was found that the pork diet supplied smaller amounts of all vitamins except thiamine. The most striking differences were in the amounts of pantothenic acid and biotin supplied by the two rations, as the one containing pork furnished only 15 per cent as much of these two vitamins as did the stock ration. Hepatic stores of thiamine, pantothenic acid and biotin were decreased in animals receiving the pork diet. The concentrations of pantothenic acid in the maternal livers were markedly reduced, although the amounts of the vitamin in the fetus appeared to be essentially normal.

Other investigations on the importance of pantothenic acid for the reproducing female and the high amounts of this vitamin present in tissues of young animals suggest that it is a critical factor in normal reproduction. Pantothenic acid deficient diets have been observed by Nelson and Evans (1946) to bring about unsuccessful reproduction in rats.

When their deficient diet was initiated on the day of gestation or approximately two weeks before mating, there was a high incidence of failure of implantation, resorptions and defective litters. The number of animals per litter was decreased as well as the birth weights of the young.

Congenital malformations in rats born of pantothenic acid deficient mothers havebeen reported by Boisselot (1948). The deficient ration fed just prior to mating caused a variety of deficiency symptoms to appear which were possibly dependent upon the vitamin stores of the female.

A relationship of pantothenic acid to metabolic processes involved in the formation of new tissue was predicted by Unna and Richards (1942). These workers noted that the need for pantothenic acid was much higher for young rats and was not related to body size or food consumption.

This vitamin has also been shown to be necessary for reproduction in chickens by Gillis, Heuser and Norris (1948). The requirement for hatchability of eggs was about five times the amount adequate for maintenance and egg production. Mortality of chicks decreased with increasing amounts of pantothenic acid in the ration of the hen.

These interesting studies, all of which suggest an unusual need for pantothenic acid during reproduction, together with the knowledge that the pregnant females which consumed the pork diet were partially depleted in this

vitamin have stimulated us to continue experimentation on the importance of pantothenic acid during the gestation period.

The investigation reported here was undertaken to study minimum needs for pantothenic acid during reproduction in the rat, and to attempt to find some relationship between a deficiency of this vitamin and the reproductive disorders observed in animals ingesting the pork diet. Two lines of attack were used in estimating minimum needs for the factor during reproduction. Excretion of the vitamin during the gestation period was followed, as a marked change in excretion of pantothenic acid should indicate a change in the animal's requirement at this time. The occurrence of pantothenic acid in placental and fetal tissues at various stages of development has been measured as a second method of estimating the increased need during gestation. Maternal hepatic and carcass tissues were likewise examined for pantothenic acid content in order that any changes in the vitamin stores of the female would be recognized.

Effects of pantothenic acid restriction on the outcome of pregnancy were studied by means of a synthetic analog (omega-methylpantothenic acid) which was added to an otherwise adequate ration, the customary stock diet. By this technique an attempt was made to reproduce some of the disorders caused by the pork diet when the animals were consuming a ration adequate in all nutritional factors except pantothenic acid.

REVIEW OF LITERATURE

Accomplishments in the chemistry and biochemistry of pantothenic acid during the period from 1933 to 1943 have been reviewed by Williams (1943), who was the first to separate this factor from "bios", a growth stimulant for yeast. The discovery of the vitamin came about during an investigation of the growth requirements of yeasts (Williams, Lyman, Goodyear, Truesdail and Holaday, 1933). Bios was found to consist of two parts which were separable by fractional electrolysis, one an acidic substance, the other basic. Both fractions were required by the Wildier's yeast, but the "Gebrude Mayer" strain of Saccharomyces cerevisiae was stimulated by the acidic substance alone. Further work showed that this acid was a very widespread if not universal constituent of living matter. Experiments were undertaken by Williams, Mosher, and Rohrman, 1936) to find out why pantothenic acid was present in tissues of plants, animals and simpler forms of life. These workers came to the conclusion that the factor was necessary for carbohydrate utilization, both aerobic and anaerobic. Pantothenic acid was necessary for fermentation, for respiration, and for glycogen storage in yeast. At this time it was suggested that there was an unknown mechanism whereby pantothenic acid was built into an essential enzyme system.

The early experiments on the effect of pantothenic acid deficiency were made without a pure source of the vitamin, and without other factors which are now known to be necessary for adequate nutrition. Some of the concentrates used were known as the "chick antidermatitis factor", the "filtrate factor", and the "liver filtrate factor". After pantothenic acid was synthesized as the calcium salt (1940) the active principle in all of these materials was shown to be pantothenic acid. Many of the pantothenic acid deficient rations were also deficient in biotin, folic acid, and perhaps B_{12}, so that the symptoms observed probably represented a complicated deficiency state.

Williams (1943) cited a list of diverse symptoms as further evidence that the vitamin had a fundamental role in cellular physiology in general. His list of symptoms of pantothenic acid deficiency in animals or fowls was as follows: dermatitis; keratitis; adrenal hemorrhage, atrophy and necrosis; cortical fat depletion; "blood caked" whiskers; depigmentation of the hair (or feathers); failure to grow; loss of hair (alopecia); thymus involution; fatty livers; stomach and intestinal ulcers; diarrhea; heart damage; kidney damage; anemia; rapid respiratory rate; rapid heart rate; prostration or coma; sudden death; convulsions; gastrointestinal symptoms; loss of viability (eggs); paralysis; myelin degeneration, sciatic nerve and spinal cord damage;

peripheral neuritis; sores about the mouth and nose; hemorrhages under the skin; severe oral lesions; abnormal cartilage (tibia); spinal curvature; increased appetite for salt. To this list has been added depression of blood lipoids of dogs, Scudi and Hamlin (1942); failure of implantation, resorptions and production of defective litters, Nelson and Evans (1946); and malformed young, Boisselot (1948).

As additional work has been undertaken to elucidate the role of pantothenic acid in nutrition certain of these deficiency symptoms have received much more attention than others. Some of the more extensively studied areas will be reviewed at this time.

Relationship between Pantothenic Acid and the Adrenals

The first report that pantothenic acid deficiency caused lesions of the adrenals was made by Morgan and Simms (1939). This investigation was made with a fullers earth treated extract of rice bran, designated as the filtrate factor. Later work proved that the active material was pantothenic acid. Histological examinations of tissues from rats fed a diet deficient in the filtrate factor showed striking and consistent atrophy of the adrenals, loss of the elastic layer of skin, failure of spermatogenesis and atrophy of hair follicles.

Hemorrhagic necrosis of the adrenal cortex was observed by Salmon and Engel (1940) in rats which were being studied

for pyridoxine deficiency. Apparently their ration was deficient in several factors. The condition did not occur in every case of pantothenic acid deficiency, but it was never present in rats which had received a source of natural or synthetic pantothenic acid. Severe necrotic changes in the adrenal cortex were observed in rats which failed rapidly, developed a severe hemorrhagic rhinitis, and in some cases passed into coma. The hemorrhagic condition was present only on the inner zone of the adrenal cortex, as the medulla was not involved.

Similar findings of adrenal hemorrhage or necrosis were reported by Daft, Sebrell, Babcock and Jukes (1940), who fed rats a B complex deficient ration containing pyridoxine but no "filtrate factor". Fractionation of the filtrate factor gave evidence that the substance preventing adrenal necrosis followed pantothenic acid, but proof that it was not an impurity was not established until synthetic pantothenic acid was obtained. The experiment was repeated using 48 young rats on a synthetic diet containing thiamine, riboflavin, pyridoxine, choline and niacin. After a period of 6 to 10 weeks symptoms of nosebleed, sticky exudate on the eyelids, depilation about the nose and mouth, and "spectacle eyes", were observed. Thirty-one of the animals were treated with pantothenic acid and sacrificed at intervals. Necrosis, atrophy and hemorrhage were found in one adrenal of one rat

in the treated group. In the untreated group, 10 out of 16 rats showed one or more of these lesions. Marked fat depletion of the adrenals was observed in 14 animals in the untreated group. Ashburn (1940) studied the histopathology of tissues of these rats and found congestion, hemorrhages, atrophy, necrosis, scarring, fibrosis, hemosiderin deposition and cortical fat depletion as independent or combined lesions in the adrenals in all 16 rats which received no pantothenic acid. Supplementation of the ration with 100 mcg. of calcium pantothenate per day markedly alleviated the symptoms. The deficiency caused no change in the spleen other than the occurrence of hemosiderin. Some animals in the untreated group displayed abnormal bone development in which the upper epiphyseal cartilage of the tibia was quite thin.

Another investigation which showed adrenal damage in rats on a purified ration was reported by Mills, Shaw, Elvehjem and Phillips (1940). Glands of animals which received no calcium pantothenate exhibited a definite pinkish or even purple color and in severe cases were greatly enlarged. Cortical necrosis of the adrenals was observed after the animals were on the deficient diet for 4 to 6 weeks. The condition was prevented by calcium pantothenate, but aggravated by choline.

That adrenals and pantothenic acid are related to hair growth and pigmentation was shown by Ralli and Graef (1943) in experiments using black rats. All animals were given

supplements of thiamine, riboflavin and pyridoxine and 1 per cent sodium chloride as drinking water. When the filtrate factor was used, it was administered in the form of a rice bran supplement. Normal rats on the ration supplemented with the filtrate factor showed bands of pigmented skin, when the hair was shaved off their backs. This coloration was absent in depleted rats, and there was also graying of fur. When the rats on the deficient diet were adrenalectomized they showed a bluish skin color at about 5 to 7 days after adrenalectomy. The color became most intense about the 16th day and then faded. New fur grown on the shaved area was not grey. When rats were transferred from the deficient diet to the supplemented ration skin and hair became normal faster in the adrenalectomized animals. The rats which were on the supplemented ration before and after being operated, showed increased hair growth and deposits of melanin after the adrenals were removed. Longer periods of maintenance on the deficient ration preoperatively decreased survival time after adrenalectomy.

Although the several experiments cited above showed that pantothenic acid deficiency caused histopathological changes in the adrenals, no evidence was given that adrenal function was impaired. Gaunt, Liling and Mushett (1946) studied the response of pantothenic acid deficient animals to water administered by stomach tube, as it is a well established finding that marked deficiencies in water diurensis and in resistance

to water intoxication result from hypofunction of the adrenal cortex in animals and man. Rats depleted of pantothenic acid showed a retention of fluid when water was given by stomach tube and had less resistance to water intoxication. Administration of either calcium pantothenate or adrenal cortical hormones improved the condition. Adrenal dysfunction due to the vitamin deficiency is suggested, although not proved as the adrenal hormones not only restored the response to normal, but actually augmented it. The effect may have been the result of a pharmacological action of these substances rather than simply the correction of pre-existing adrenal dysfunction.

The effect of pantothenic acid deficiency on tissue changes and sodium balance in rats was studied by McQueeney, Ashburn, Daft and Faulkner (1948). Retention of sodium was essentially the same for the pantothenic acid deficient and control animals. Treatment of depleted rats with 1 mg. of calcium pantothenate or 1.5 mg. of desoxy-corticosterone acetate had no effect on sodium and potassium balances. Histopathological changes were noted in adrenal glands, thymus, lymph nodes, bone and marrow.

Deane and McKibben (1946) classified the changes in adrenals brought about by pantothenic acid deficiency as an "alarm reaction". They made a detailed study of the amounts of ketosteroids present in the adrenal cortices during the development of pantothenic acid deficiency, and found that

the zonal reticularis and fasciculata were progressively depleted of the hormones by the end of 6 weeks. They interpreted the symptoms as indications that the pantothenic acid deficiency acts as a severe "alarming agent", causing the release of pituitary adrenotropin which stimulates the adrenal to enlarge and increase its secretion of corticosterone. The activity of the adrenal is stepped up to the point that it becomes exhausted of corticosterone.

Survival of adrenalectomized rats was significantly increased when the diet was supplemented with very large doses of pantothenic acid and 1 per cent sodium chloride used as drinking water (Ralli, 1946). After 30 days on the pantothenic acid deficient diet and the removal of the adrenals, rats receiving 4 mg. or more of pantothenic acid daily survived for prolonged periods (Dumm and Ralli, 1948). The adrenalectomized rats receiving large amounts of pantothenic acid were in unusually good physical condition 100 days after the operation. After the stress of swimming there was less decrease in blood sugar values for these rats than for intact animals on the stock diet. The effect of pantothenic acid in increasing survival time of these animals may be a protection from the disturbance in carbohydrate metabolism which usually follows adrenalectomy. Ralli and her associates suggest that another effect of pantothenic acid and sodium chloride administered together may be through the maintenance of water balance.

In further investigations of the role of pantothenic acid in increasing the survival time of adrenalectomized rats, Dumm and Ralli, (1949) have studied the excretion of pantothenic acid and ascorbic acid. Adrenalectomy had no effect on the amount of pantothenic acid excreted when the daily intake was 4 mg. The adrenalectomized rats receiving pantothenic acid excreted less ascorbic acid than their controls, but this lower excretion apparently was not detrimental to prolonged survival.

Interest aroused by the work of Dougherty and White (1944) on the role of the adrenal cortex in controlling the number of lymphocytes in the circulating blood, stimulated Dumm, Ovando, Roth and Ralli, (1949) to investigate the effect of diets adequate and deficient in pantothenic acid on the white cell and lymphocyte counts following stress. Rats were injected with adrenocorticotropic hormone or were forced to swim for 25 minutes, and cell counts were made at intervals thereafter. A typical lymphopenia was observed within 2 hours following either type of stress in rats on the complete diet. This response was partially abolished in rats on the pantothenic acid deficient diet. These results give further evidence of the importance of pantothenic acid in maintaining normal adrenals.

A relationship between the physiological integrity of the adrenals and normal reproductive performance has been

suggested by Molsberry, (1943) an earlier investigator in this laboratory. Molsberry observed that lesions in the adrenals were detectable histologically in all rats which developed toxemia of pregnancy when fed the experimental diet containing pork. There was no evidence of abnormality in the adrenals of animals ingesting the pork ration which did not exhibit the pregnancy disorder or in the stock animals.

Relationship of Pantothenic Acid to Reproduction in Animals

In one of the early attempts to formulate a ration for rats which contained only synthetic forms of the B-vitamins, Jukes (1940) studied the effect of omitting pantothenic acid on reproduction. Of the nine females fed the deficient ration, three rats resorbed their fetuses while five produced young which died within 4 days of birth. Additional evidence that pantothenic acid is related to normal reproduction was presented by Figge and Allen, (1942). Genital atrophy was observed in female rats maintained on a pantothenic acid deficient diet. The vaginal orifice of such animals did not open until the animals were 88 days old in comparison to the usual age of 40 to 45 days when pantothenic acid was supplied. Deficient animals had tiny and "anemic" uteruses and extremely underdeveloped ovaries.

Taylor, Pennington, and Thacker (1943) have reported that reproduction in rats and mice could be improved if commercial dog chow was supplemented with pantothenic acid. When the commercial ration which provided 14 mcg. of pantothenic acid per gm. of feed was supplemented with 100 mcg. of calcium pantothenate administered by stomach tube each day, the average number of young produced increased from 5.4 to 6.7. It was suggested that extra pantothenic acid either stimulated ovulation or enabled more of the fertilized ova to survive to birth. The investigators favored the latter explanation.

Nelson and Evans (1946) observed reproduction in rats fed pantothenic acid deficient diets before and at intervals during gestation. Two basal diets, differing in the amounts of B-vitamins and the type of salt mixture were tested. Both rations contained the following ingredients: alcohol extracted casein, 24; sucrose, 64; hydrogenated cottonseed oil, 8; and salts, 4. All rats received weekly a fat soluble vitamin mixture which furnished a minimum of 400 U.S.P. units of vitamin A; 58 A.O.A.C. Chick units of vitamin D; 3 mg. synthetic alpha-tocopherol; and 325 mg. of corn oil. One deficient diet (841) contained McCollum salts No. 185 and the following B-vitamins per kg. of ration: thiamine HCl 2 mg., pyridoxine HCl 2 mg., riboflavin 4 mg., p-aminobenzoic acid 5 mg., nicotinic acid 10 mg., inositol 200 mg., and choline HCl 0.5 gm. The corresponding control diet (831) contained 28 mg.

calcium pantothenate per kg. A second deficient diet (846) contained an improved salt mixture, No. 4 and the following levels of B-vitamins per kg. of ration: thiamine HCl 5 mg., pyridoxine HCl 5 mg., riboflavin 10 mg., p-aminobenzoic acid 10 mg., nicotinic acid 20 mg., inositol 400 mg., and choline HCl 1.0 gm. Control diet (836) contained 50 mg. calcium pantothenate per kg. of ration.

Initiation of the deficient diets to a portion of the animals on the 13th day of gestation caused no difference in reproductive performance between these animals and the control groups. However, marked upsets during reproduction occurred when the deficient experimental diets were started on the day of mating. Resorptions occurred in approximately one-third of the rats receiving either deficient ration. The average weight of the young was significantly decreased; in the rats on diet (841) the average number of young per litter was decreased. Two of the seven litters produced on diet (846) were found in the uterus on autopsy and consisted of some living fetuses, some dead, and others in the process of resorption. The reproductive disorders were more severe when the females were transferred to the deficient diets 15 days before mating. Failure of implantation occurred in about one-third of the deficient rats. In animals on diet (841), one-half of the implantations underwent resorption while all of the implantations on diet (846) were resorbed. The 4 litters produced on diet (841) were small in number and birth

weight. When the deficient animals were changed to the corresponding control diets on the first day of gestation, reproduction was essentially normal. Restriction of calories to 69 per cent by paired feeding did not cause reproductive disturbances in animals receiving the control diets. Aggravation of the reproductive disorders, observed when the higher quantities of vitamins were fed may have been due to greater growth stimulation, thus increasing the pantothenic acid requirement. Possibly the presence of largeramounts of one of these factors accentuated pantothenic acid deficieney.

Congenital malformations of the young occurred when rats were fed a pantothenic acid deficient diet preceding and during pregnancy (Boisselot, 1948). Normal females were maintained on a pantothenic acid deficient diet until some of the group became depleted, as evidenced by irregularities in the estrus cycle. At this time an attempt was made to mate all the animals with normal males. Failure of implantation occurred in the females which had shown the symptoms of deficieney.

Among the others which became pregnant there were many which resorbed their young and lost weight by the 14th or 15th day. In the case of the less deficient animals gestation advanced to term but the young were abnormal. Control rats receiving the same ration supplemented with calcium pantothenate produced normal litters.

The young born to females restricted in pantothenic acid showed various degrees of edema which caused a loosening of the skin which was especially marked in the region of the head and the thorax. There were reported disturbances in the central nervous system and circulatory trouble which was characterized by paleness of the limbs contrasting with the appearance of hemorrhagic zones coincident with alterations in the digits. Although the level of the vitamin in the diet was below that necessary for the production of normal young, the authors reported that no deficiency symptoms were apparent in the mothers.

Several experiments have shown that pantothenic acid is necessary for satisfactory reproduction in poultry. Bauernfeind and Norris (1939) fed a heated diet to pullets and found that hatchability of the eggs was decreased to 2.7 per cent. Improvement in hatchability was brought about by supplementation of the heated ration with 5 per cent anti-dermatosis vitamin concentrate and 5 per cent whey adsorbate. Pearson, Melass and Sherwood (1945) observed that the pantothenic acid content of eggs reflected the diet of the hen and that the quantity of vitamin in the tissues of the newly hatched chick was equal to that present in the egg.

The pantothenic acid requirement of hens to insure good hatchability of eggs was estimated to be about 800 mcg. per 100 gm. of diet, according to experiments conducted by Gillis, Heuser and Norris (1947, 1948). Only 150 mcg. of the vitamin

per 100 gm. of diet was adequate for maintenance and egg production but after 10 weeks on this intake hatchability of the eggs was reduced to zero. Mortality of chicks from hens who had received 350 mcg. of pantothenic acid per 100 gm. of food for 11 weeks was 50 per cent in 4 weeks. There was no mortality among chicks from hens which received 650 mcg. per 100 gm. of diet. However, these chicks did not grow as well as those produced from hens receiving higher levels of the vitamin.

Pantothenic Acid Requirements of Young Animals

One reason for believing that pantothenic acid is of special importance during reproduction is the high requirement of the vitamin observed for young animals. Weanling rats on a pantothenic acid deficient diet ceased to gain weight in 3 weeks and survived only 25 to 60 days (Unna, 1940). The daily requirement for young rats was estimated to be 80 to 100 mcg. (Unna, 1940; Henderson, Mc Intire, Waisman and Elvehjem, 1942). The daily maintenance dose decreased with progressing age of the animal; the adult rat was found to require only 25 mcg. of the vitamin per day (Unna and Richards, 1942). These investigators state that the change in needs was not the result of storage during the growth period, as the requirement changed even when rats were given only the minimum amount of the vitamin necessary to

support growth. The requirement showed no relation to body size or food consumption. The suggestion was made that pantothenic acid had an entirely different role in metabolism from the other vitamins of the B complex, and that it may be connected with metabolic processes involved in the formation of new tissue.

Survival time of pups on a pantothenic deficient ration was only 1 month while adult dogs lived for 6 months or longer on the same ration (Silber, 1944). Weanling silver and red fox pups stopped growing after 2 to 3 weeks when pantothenic acid was omitted from the ration (Schaefer, Whitehair and Elvehjem, 1947). Two animals died after 26 and 27 days on the experiment. The requirement of pantothenic acid for fox pups of this age was estimated to be above 0.25 mg. and below 1.5 mg. per 100 gm. of ration.

Gillis, Heuser and Norris (1948) suggested that the rapidly growing chick needs approximately 600 mcg. of pantothenic acid per 100 gm. of food, or 4 times the maintenance requirement of the adult fowl. An intake of 900 mcg. per 100 gm. of diet was recommended by Hegsted and Riggs (1949) as the optimum level of the vitamin for young growing chicks. These authors suggest that the concentration of pantothenic acid of the liver is a less sensitive criterion for judging adequacy of the diet than growth. The quantity of pantothenic acid in the livers of chicks remained normal when the

birds consumed rations containing 130 to 300 mcg. of calcium pantothenate per 100 gm; however, growth was depressed.

The young duck appeared to be especially sensitive to pantothenic acid deficiency (Hegsted and Perry, 1948). Birds failed to grow 2 or 3 days after they had been transferred to a ration deficient in this vitamin and death occurred within 4 to 7 days. The growth requirement was estimated to be 1100 mcg. per 100 gm. of food for this fowl. Turkey poults have likewise been shown to require large amounts of this factor according to the work of Kratzer and Williams (1948).

It is obvious that there are many indications that pantothenic acid is required in unusually high concentrations during early life but as yet little is known of its purpose in young tissue.

Relationship between Pantothenic Acid and Protein

A relationship between pantothenic acid and protein has been proposed by several workers although there is no proof that the interrelationship is a direct one. Alterations of the proportion of the protein of the diet automatically change the amounts of fat and carbohydrate or both so that it is difficult to assess the importance of one constituent.

Wright and Skeggs (1946) have been interested in this problem, and have observed that pantothenic acid excretion in the feces paralleled the protein level of the diet. When only

5 per cent protein was fed, symptoms of B complex deficiency became evident. The high renal clearance of administered calcium pantothenate and the usual occurrence of pantothenic acid in a combined state suggested to these authors that during protein deprivation a substrate for combination with the vitamin was not available.

Nelson and Evans (1945) have investigated this interrelationship for some period of time. The California workers observed that when rats were fed two rations deficient in pantothenic acid, one containing 64 per cent casein and the other 24 per cent, better growth and increased survival time occurred in the group ingesting the higher level of protein. The investigators gave several possible explanations for the superiority of the high protein diet. Decreasing the level of carbohydrate was thought possibly to have produced the effect. Components of casein were suggested as being important in producing the beneficial response.

In a continuation of the investigation of the pantothenic acid sparing action of protein, Nelson, van Nouhuys and Evans (1947) determined both fecal and urinary excretion of the vitamin when the two levels of casein were fed. Urinary excretion of pantothenic acid was equal to 6.0 mcg. per day for animals fed the 64 per cent casein ration and 0.9 mcg. per day for the 24 per cent level. There were no differences in fecal excretion of pantothenic acid in the two groups prior

to the 90th day at which time the high protein group excreted somewhat more pantothenic acid. As the urinary excretion of the vitamin was higher on the high protein diet, there was no indication that the sparing action resulted from decreased loss of the vitamin in urine. It was suggested that some component of casein may replace or be converted into pantothenic acid in a manner similar to the tryptohane-niacin interrelationship. Substitution of washed beef fibrin for the casein was made to determine whether or not the effect of casein was due to its content of pantothenic acid (Nelson and Evans, 1947). Fibrin showed an average pantothenic acid content of 0.5 mcg. per gram whereas casein contained 1.5 to 1.8 mcg. per gram. Four groups of rats were fed diets containing the two sources of protein at levels of 24 and 48 per cent. Deficiency symptoms were accentuated in the animals receiving the smaller source of protein. Doubling the amount of fibrin resulted in increased growth and survival. Greater differences were apparent between the groups on the two levels of fibrin than had been observed for the two levels of casein.

Another approach to the relationship between pantothenic acid and protein was made by adding single amino acids equivalent to 10 per cent or to 30 per cent of casein to a pantothenic acid deficient diet containing 24 per cent casein (Nelson and Evans, 1949). Methionine had a marked sparing action, improving both growth and survival of the deficient

rats. Cystine and possibly glutamic acid and glycine appeared to have some sparing action. There was no change in the urinary excretion of pantothenic acid caused by the addition of these amino acids.

The relationship of the concentration of coenzyme A in the liver to liver protein was studied by Harkness, Seifter, Novic and Muntwyler (1949). Rats receiving adequate amounts of pantothenic acid were given diets in which protein was restricted. After 3 weeks the amounts of coenzyme A in the livers of the experimental animals were decreased in comparison with pair fed controls. However, when the coenzyme A values were calculated on the basis of liver nitrogen, there was no appreciable difference between the two groups. The concentration of coenzyme A was directly proportional to the nitrogen content of the liver. The decrease in coenzyme A content in the liver was produced on a diet supplying approximately twice the amount of pantothenic acid usually allowed for adequate diets.

An increased need for pantothenic acid by adult rats was observed when animals were induced to grow again by injections of the anterior pituitary growth hormone (Lotspeich, 1950). Two groups of adult animals were placed on high fat and high carbohydrate diets for two weeks, and were then given daily injections of the growth hormone. There was a weight gain during the first week, but in the second week it was observed

that the animals were not gaining as rapidly as before and were not consuming their ration. Symptoms of a pantothenic acid deficiency became evident, especially in the animals on the high fat diet. An examination of the diet revealed that pantothenic acid had been omitted, and injections of calcium pantothenate caused a dramatic increase in weight gain. It is extremely difficult to develop signs of pantothenic acid deficiency in adult animals, but apparently in this case the rapid growth following hormone injections increased the requirement for the vitamin. The investigator speculated that the increased need for pantothenic acid was due to renewed growth and the resulting need for greater protein synthesis. He stated that since coenzyme A was known to be necessary for certain acetylation reactions and that this process represented the synthesis of a peptide bond that the need for pantothenic acid appeared to be due to the increased synthesis of protein as a result of renewed growth.

Miscellaneous Papers Concerned with Pantothenic Acid Deficiency

The ubiquitous presence of pantothenic acid in living matter suggested even in the early experiments that this factor played a fundamental role in metabolism. Since the material is present in every living cell it is not surprising that reports of gross symptoms of deficiencies have been numerous

and diverse. Changes in the adrenals, reproductive failure and mortality of young associated with pantothenic acid deficiency have been discussed, however, several other papers will be mentioned briefly since they may be equally important in expanding our information concerning the role of pantothenic acid.

In some of the first experiments when the filtrate factor was being investigated, it was observed that a deficiency of this material caused greying of fur of black or hooded rats (Morgan and Simms, 1940; Oleson, Elvehjam and Hart, 1939). Concentrates from liver, rice bran, yeast, crude cane molasses or alfalfa cured or prevented the onset of the condition.

Rations deficient in pantothenic acid caused a marked depression in the concentration of blood lipoids in dogs (Scudi and Hamlin, 1942). Blood cholesterol, cholesterol esters, lipoid phosphorus, and total lipids were all lowered. The deficiency was more critical in young animals than in adults. Blood levels of lipoids were increased by as little as two daily doses of the vitamin, although fatty livers were not prevented. Since livers were normal when daily supplements of 5 gm. of dried beef liver were administered, but not with calcium pantothenate, it was assumed that some other factor was involved.

Marked changes in hematopoiesis were observed in rats on a pantothenic deficient diet (Ashborn, Daft and Faulkner,

1947). Granulocytopenia and anemia developed singly or in combination. Lymphoid tissue, spleen, thymus and cervical nodes showed atrophy of a variable degree, although the condition was most severe in the thymus. Adrenals were depleted of lipoids and occasionally showed hemorrhage and necrosis.

Shaw and Phillips (1945) used a heated diet composed of natural foodstuffs and a partially synthetic sucrose ration deficient in pantothenic acid in studying neuropathologic changes in chicks. The heated ration was deficient in more than one factor as the rate of growth was below maximum even when calcium pantothenate was added to the basal diet. A folic acid deficient ration was devised also to eliminate the possibility of that deficiency being involved in the condition of the animals. Severe myelin degeneration in the spinal cord occurred in chicks fed the heated ration alone. This was entirely prevented when calcium pantothenate was administered. The addition of a solubilized liver fraction supplying the folic acid complex brought about a very appreciable increase in the rate of growth, although the chicks still showed signs of a biotin deficiency. There was no evidence of lesions in the spinal cord or in the sciatic nerves when the biotin deficient chicks were receiving pantothenic acid. On the sucrose ration the degeneration of the spinal cord was of the same type as that observed on the heated ration. This change was prevented by the addition of 10 to 20 mg. of

calcium pantothenate per kilogram of food. There was no evidence of myelin degeneration in the sciatic nerve. In the chicks on the folic acid deficient ration there were no lesions of the spinal cord, but mild changes in the sciatic nerve were detectable. It was concluded that myelin and axon degeneration of the spinal cord was due to the deficiency of pantothenic acid and not to a lack of any other nutritional factor.

The susceptibility of chicks to histopathological changes of the spinal cord was confirmed in a similar investigation using a partially synthetic ration (Ram, 1949). External manifestations of deficiency became evident when the dietary pantothenic acid was decreased below 550 mcg. per 100 gm. of ration. Demyelinization of the spinal cord extended to all tracts dealing with motor apparatus in all sections of the brain,--the forebrain, brain stem and cerebellum. Peripheral nerves were unchanged.

Recently Bowles, Hall, Sydenstricker and Hock (1949) have reported that corneal changes develop in rats restricted in pantothenic acid. Weanling animals placed on a ration deficient in this factor died before any changes in the eyes could be detected by a biomicroscope. However, if the animals were transferred to the deficient diet at 41 to 61 days of age more than half of the animals showed changes characterized by heavy vascularization, thickening, and opacity of the cornea.

Inhibition of endochondral ossification of the tibia in pantothenic acid deficiency has been observed in mice (Levy and Silberberg, 1946), and in rats (Nelson, Sulon, Becks, Wainwright and Evans, 1950). In both species tibia length was shortened, and epiphyseal width decreased. Later stages of the deficiency in the rat showed the onset of calcified sealing off of bone, indicating growth had stopped.

The many disorders reported in animals or fowl partially depleted of pantothenic acid seem unrelated and difficult to explain at this time. However, as has proved the case for other vitamins in the past, diversity of deficiency symptoms disappears when the role of the factor in metabolism is more clearly understood.

Coenzyme A

Pantothenic acid was first demonstrated a vital component of an enzyme system by Lipmann and his associates in 1947 (Lipmann, Kaplan, Novelli, Tuttle and Guirard, 1947). These workers liberated bound pantothenia acid from an active substance which they called coenzyme A by prolonged treatment with clarase and papain. More recently it has been shown that complete liberation of the vitamin requires enzymatic treatment with phosphodiesterase and an unidentified enzyme present in pigeon or chicken liver (Lipmann, Kaplan and

Novelli, 1947). The Boston workers have suggested that coenzyme A is a constituent of all living cells and that the pantothenic acid present in tissues is there mainly in the form of the coenzyme.

Decreased ability to acetylate p-aminobenzoic was observed in pantothenic acid deficient rats by Riggs and Hegsted, 1948. The acetylation curve reflected the coenzyme A content of the liver during pantothenic acid deficiency. When 1 mg. quantities of calcium pantothenate were injected into deficient rats an immediate response was encountered and normal acetylation was possible. Similarly pantothenic acid deficient rats were observed to be unable to acetylate sulfanilamide at a normal rate (Shils, Seligman and Goldwater, 1949). Changes in acetylation rate occurred when young male rats were restricted in pantothenic acid for 3 weeks. Older male animals maintained on the deficient diet for longer periods of time likewise lost the ability to acetylate sulfanilamide. Injections of calcium pantothenate had little effect, but when the animals were transferred to the control diet for one week improvement in acetylation was evident.

The widespread occurrence of coenzyme A inall living matter suggested that its role in metabolism exceeded that of simply acetylating aromatic amines (Novelli and Lipmann, 1947). A fuller understanding of its metabolic action was forthcoming when these workers observed that coenzyme A

played an important part in the reaction of acetate with adenylpyrophosphate to yield a compound having the properties of acetyl phosphate. It has been suggested that possibly coenzyme A plays a part in the metabolism of 2 carbon compounds in general.

Enrichment of yeast with pantothenic acid raised the coenzyme A content from approximately 100 to 500 units per gm. of dry material. This yeast respired acetate and ethanol twice as rapidly as did the unenriched samples (Novelli and Lipmann, 1947b). With ethanol as the substrate more than half its equivalent accumulated as acetic acid in the deficient yeast while only traces were detectable in the coenzyme A-rich sample. The authors cite this experiment as support for the view that coenzyme A is concerned primarily with acetate removal, presumably with its condensation with oxalacetic.

More recently Soodak and Lipmann (1948) have observed that acetoacetate synthesis is inhibited by sulfanilamide.

The relationship between tissue coenzyme A and pyruvate metabolism in ducks and rats at various stages of pantothenic acid depletion has been studied by Olson and Kaplan (1948). Young rats fed a pantothenic acid free diet were sacrificed at 3, 5, and 9 week intervals while one week old ducklings were killed on the 5th, 10th and 15th days of pantothenic acid restriction. Coenzyme A determinations were made on

liver and heart tissue and pyruvate utilization was measured for slices of these two organs. As the vitamin deficiency progressed, coenzyme A activity of the tissues was reduced and the metabolism of pyruvate by tissue slices was diminished. Addition of pantothenic acid to heart and liver slices caused small but definite increases in coenzyme A content. Normal tissues, however, did not synthesis additional coenzyme A when pantothenic acid was added.

The metabolism of radioactive acetate and pyruvate by cardiac muscle from normal and pantothenic acid deficient ducklings was measured by Olson and Stare (1949). In the slice but not in the homogenate considerable amounts of added pyruvate were metabolized to non-lactate products without the loss of carbon dioxide. The oxidation of added pyruvate in slices of ventricle from deficient ducks was essentially normal, but was depressed in the homogenate. The oxidation of acetate was reduced in both slices and homogenates of tissue from the deficient ducks. Another series of experiments conducted by the same group of workers revealed that there was decreased synthesis of citrate from pyruvate and fumarate by homogenates of heart ventricle from pantothenic acid deficient ducklings (Olson, Hirsch, Richards and Stare, 1949). These observations suggest that coenzyme A is involved in the initial condensation of the tricarboxylic acid cycle.

Novelli and Lipmann (1950) have studied acetate oxidation in yeast which was known to proceed through the citric acid cycle. Coenzyme A enriched yeast was prepared by incubating a sample with pantothenic acid in a glucose-phosphate medium. Enrichment increased the number of coenzyme A units per gm. from 100 to 370 and doubled the rate of acetate oxidation. Oxygen and acetate consumption was lowered in the coenzyme A deficiency although the ratio of oxygen to acetate remained the same for both samples. Complete oxidation was indicated in both cases. These data have indicated to the workers that it is the rate of primary attack on acetate which is depressed during a deficiency of coenzyme A, and that condensation of acetate and oxalacetate to form citric acid involves coenzyme A.

These recent investigations which add pantothenic acid to the list of vitamins essential for enzyme catalyzed reactions in carbohydrate utilization prove that the vitamin has a place of importance in metabolism. The relationship between the importance of pantothenic acid in carbohydrate metabolism and the high requirement for the formation of new tissue is not understood.

EXPERIMENTAL PROCEDURE

The present investigation was undertaken to study two main questions, first the quantitative pantothenic acid requirements of the rat during pregnancy and second the feasibility of inducing toxemia of pregnancy in rats by tying up the pantothenic acid of a ration known to support satisfactory reproduction. The experimental work has taken many ramifications as observations were encountered which seemed interesting to study further. To clarify the procedure followed during the investigation it has seemed most satisfactory to divide this description into several distinct sections.

Procedure for Studying the Pantothenic Acid Requirement of the Rat during Reproduction as Based on Tissue Analyses

The deposition of pantothenic acid in maternal and fetal tissues of rats during reproduction was studied in an attempt to estimate needs for the vitamin above that necessary for the maintenance of the adult organism. This study was similar to an investigation by Barrett (1950) on thiamine and riboflavin requirements during reproduction in rats, and employed the same animals. Females of weanling age were maintained on the stock ration and were mated when approximately 70 days old (4 weeks after the opening of the vaginal orifice).

Shortly after the birth of the first litter by each female, the young rats were examined critically for evidence of defects, and birth weights were recorded. These first litters were discarded since the present investigation was planned to determine reproductive needs uncomplicated by lactation. Twenty-four hours after the removal of the first litter, females were remated, and observations on the vitamin content of tissues were made during the time rats were producing their second litters.

It was planned that three females would be sacrificed at given intervals during second pregnancies in following the amount of pantothenic acid present in fetal and placental tissues at various stages of pregnancy. Maternal tissues were also assayed for vitamin potency so that information would be known about possible fluctuations in maternal stores throughout pregnancy. When the females were in a mating stage and the male was introduced into the cage, vaginal contents were examined every four hours so that the time of insemination could be established accurately. Presence of sperm or the copulation plug were used as criteria of the initiation of pregnancy. Groups of animals were sacrificed on the 6th day of the gestation period, on the 10th day and at 1-day intervals subsequently to parturition. If the females were producing less than 9 young at the time they were sacrificed, the rat and her litter were discarded and an additional female was assigned to the particular unit. A total of 15 groups of

animals were included in this portion of the study. This number included three rats which were not pregnant but which had delivered first litters, three females which had just produced their second young and 13 groups of animals which were in various stages of the pregnancy period. Details of the procedure followed in preparing the maternal and fetal tissues for vitamin assay and the method of analyses will be presented later.

Procedure for Studying Urinary Excretion and Tissue Stores of Pantothenic Acid as Related to Intake of the Vitamin

Experiments were designed in which the urinary excretion of pantothenic acid was measured throughout pregnancy in rats which ingested the customary stock diet and in the same ration enriched with calcium pantothenate. Effects of the various feeding regimes were evaluated by the outcome of pregnancy, the amounts of the vitamin excreted by the kidney and the concentration of pantothenic acid in hepatic and carcass tissues of the adult rat and the entire newborn. In these metabolism experiments only urinary excretion of pantothenic acid was considered, since it had been shown earlier by Henderson, Mc Intire, Waisman and Elvehjem (1942) that the fecal excretion of pantothenic acid was not related to the dietary intake of the vitamin.

Observations were first made on rats receiving the usual stock ration, as it was assumed that this diet supplied adequate amounts of all nutrients in view of the successful reproductive performance of animals in the breeding colony. However, when the first series of experiments revealed that the quantity of pantothenic acid excreted by the kidney decreased markedly during the latter part of pregnancy, it seemed advisable to investigate the value of enriching the ration with calcium pantothenate. This was done by adding calcium pantothenate at the beginning and on the 16th day of pregnancy. Rations containing extra pantothenic acid were prepared by adding 20 or 50 mg. of calcium pantothenate per kilogram of basal ration. The crystalline vitamin was weighed on an analytical balance and was thoroughly incorporated into a small amount of casein of the ration.

Since several investigators have reported an increased need for pantothenic acid in young tissue, a small number of rats were sacrificed at 6 and 10 weeks of age so that information might be gained about the early stores of this vitamin in animals used in the present investigation. The following experimental groups were included in this phase of the study:

Number of Rats	Ration	Age when sacrificed
4	Stock ration	6 weeks
4	Stock ration	10 weeks
8	Stock ration	13 weeks

Number of Rats	Ration	Age when sacrificed
9	Stock ration	13 weeks
2	Stock ration plus 50 mg. calcium pantothenate per kg. ration from initiation of pregnancy	13 weeks
4	Stock ration plus 50 mg. calcium pantothenate per kg. ration added on the 16th day of pregnancy	13 weeks
2	Stock ration plus 20 mg. calcium pantothenate per kg. ration added on the 16th day of pregnancy	13 weeks

Procedure Followed in Determining the Influence on Reproduction of a Pantothenic Acid Deficiency Induced by Feeding Omega-Methylpantothenic Acid

The reproductive disorders which appeared in females maintained on the ration containing pork and yeast as the principle sources of the B-vitamins and protein have been erratic during recent years, as has been mentioned earlier. Therefore it seemed desirable to attempt to produce a more dependable means of precipitating the syndrome of toxemia if any amount of information concerning the effect of nutrition upon its incidence, severity, or correction were to be gained. As a synthetic ration known to support reproduction satisfactorily had not been formulated for the rat, the deficiency was created by adding a pantothenic acid analog, omega-methylpantothenic acid[1], to the stock diet. This compound has

[1]Kindly supplied by Dr. Max Dunn, Department of Chemistry, University of California, Los Angeles, California.

been shown to exhibit competitive inhibition of pantothenic acid in lactic acid bacteria and mice (Drell and Dunn, 1948, 1949). Two lots of analog were used, one of 95.0 per cent purity and the second 93.1 per cent pure. Allowances were made for impurities which were largely excess ethanol and lactone. The analog was incorporated into the ration in a manner similar to that described for the addition of the vitamin.

The analog was first fed at levels of .15 and .30 per cent of the ration. These concentrations were based on suggestions from Dr. Dunn[1] who had estimated that 15 or 20 mg. of the analog per rat per day would reduce the pantothenic acid activity of the stock ration into the range of the pork diet, which had induced the syndrome of toxemia. As these quantities of inhibitor produced complete resorption of the litters at an early portion of pregnancy, the amount of analog was decreased in later work. A level of .05 per cent was tried next and finally the inhibitor was increased to .075 per cent.

Rations containing both inhibitor and vitamin were tested in order to prove that the activity of the analog was competitive rather than toxic. An attempt was made to produce an acute pantothenic acid deficiency by feeding the ration containing the analog from the 16th day of pregnancy,

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[1]Personal communication.

during the time of rapid increase in mass of fetal tissue. A total of 26 animals received omega-methylpantothenic acid. These females were divided into the following experimental groups:

Number of rats	Ration
4	Stock ration plus .3 per cent analog
4	Stock ration plus .15 per cent analog
4	Stock ration plus .15 per cent analog started on the 16th day of pregnancy
2	Stock ration plus .15 per cent analog plus 20 mg. calcium pantothenate per kg. ration from initiation of pregnancy
4	Stock ration plus .15 per cent analog plus 100 mg. calcium pantothenate per kg. ration from initiation of pregnancy
3	Stock ration plus .075 per cent analog
3	Stock ration plus .05 per cent analog for 3-5-7 days during pregnancy and then increased to .075 per cent of the analog
2	Stock ration plus .05 per cent analog

Selection of Animals

Twenty-eight day old female albino rats representing highly inbred Wistar stock were used throughout the study. At weaning these animals weighed between 48 and 56 grams.

The Ration

The ration used during the experiment was a modification of the stock diet suggested by Steenbock (1923); it was chosen because of an abundance of evidence that is supported successful reproductive performance in the breeding colony and because some question still exists concerning the adequacy of highly synthetic rations. The composition of the diet was as follows:

Yellow cornmeal	56.0
Linseed meal	16.0
Casein	5.0
Alfalfa meal	2.0
$CaCO_3$*	0.5
NaCl	0.5
Yeast	9.5
Yeast (Irradiated)	0.5
Wheat germ	10.0
Dried whole milk	33.0

*Trace elements added: KI, $MnSO_4$, $Al_2K_2(SO_4)_4$, $CuSO_4$

Supplements of 5 gm. of raw beef and 10 gm. of carrots were fed on alternate days. Two drops of cod liver oil were fed directly to the animals daily. Distilled water and the stock ration were supplied ad libitum.

Every effort was made to provide the animals with a uniform ration. Four groups of animals have been included in the experiment and preceding each anticipated study sufficient ration to cover the needs of the entire section of work was prepared and refrigerated. Enough beef round for one series of animals was trimmed of fat and ground thoroughly. Portions

of 5 gm. each were weighed, wrapped individually, and stored at 20° F. until needed. Carrots for the period were washed, wrapped in heavy paper, and stored at 40° F. Assays for pantothenic acid content were made on each lot of Steenbock ration, meat and carrots.

Metabolism Experiments

Urine collections were made from one series of rats beginning 24 days before mating and lasting throughout pregnancy. Only the gestation period was studied in two later series of animals. In each case, however, non-pregnant animals of the same age were observed during a comparable period of time. Collection periods were 4 days in length until the 8th day of pregnancy, after which time they were shortened to 2 days in order to observe more exactly the time of changes in excretion of pantothenic acid.

Metabolism cages were round and were made of galvanized wire mesh. They were suspended over pyrex pie plates by means of metal strips bent to hook under the bottom rim of the cage and over the edge of the plate. Cage bottoms were of half inch mesh and were raised to reduce coprophagy. Feces were separated from urine by a fine wire screen cut slightly larger than the bottom of the pyrex plate so that it was supported about a half-inch from the bottom by the sloping sides of the plate. Ointment jars with metal covers

in which holes of one inch diameter had been cut were used as food cups. These were wired to the sides of the cages. Scattering of the ration was minimized by these precautions.

The supplement of meat was usually consumed soon after it was placed in the cage. Carrots were sometimes broken into small pieces which fell through the mesh of the cage and out of reach of the rat. When these pieces were again offered to the rat, they were eaten even though dehydration had taken place. In order to be sure that all of the supplements were consumed, meat was always fed on the last day of the collection period, at which time particles of carrot left from the preceding day were placed in the food cup. Each day the wire screen under the cage was removed long enough for the removal of feces and any small pieces of food.

Feces and small amounts of dry ration spilled on the plate were discarded before urine collections were made. No special precautions were used to prevent bacterial decomposition of urine during the metabolism periods, as it was observed that rapid evaporation from the surface of the plate kept the material fairly dry.

Urine was collected by washing the cage bottom, wire screen, and plate using a rubber policeman and a fine stream of distilled water from a siphon bottle. Washings were filtered through cotton into a volumetric flask. The process was repeated 3 times and the pooled washings were made to a

volume of 250 ml. Urines were transferred to bottles containing a small amount of toluene and were then stored in a refrigerator until pantothenic acid determinations were completed.

Preparation of Animal Tissues and the Ration for Vitamin Assays

The procedure followed for the preliminary preparation of animal tissues is described in detail by Barrett (1950). These tissues have included the maternal liver and carcass, the fetuses, and in certain instances the placentae. Prior to the 13th day of pregnancy in the case of experimental animals being sacrificed at frequent intervals during the gestation period, fetal tissues could not be separated satisfactorily from the placentae so that the uterus and its contents were prepared together.

Since considerable interest has been aroused by the work of Novelli, Kaplan and Lipmann (1949) and by King, Locher and Cheldelin (1948) regarding the form in which pantothenic acid occurs in animal tissues, hepatic tissues of certain of the pregnant rats and their fetuses have been prepared so that both free and bound pantothenic acid could be measured. These tissues were taken from animals which had had access to the stock ration or this diet supplemented with either calcium pantothenate or the vitamin inhibitor.

Following an injection of sodium pentobarbitol (Nembutal) the various tissues were excised, weighed, and fragmented by mixing in a Waring blendor. Acetate buffer (pH 4.6-4.8) was added and the tissues were diluted to a known weight. Representative liver and fetal tissues were prepared for assay of free pantothenic acid by pouring a portion of the homogenate into an equal volume of boiling water. Heating was continued for 1.5 minutes in order to inactivate autolytic enzymes which were known to release bound pantothenic acid. The tissues were later transferred to one-half pint jars, covered with a thin layer of toluene and stored at 20° F.

Frozen tissues were thawed at room temperature and again blended in a Waring blendor in preparation for sampling. Aliquots for both free and total pantothenic acid assays were weighed on a small torsion balance. Samples for free pantothenic acid assay were adjusted to pH 6.6 and autoclaved at 15 pounds pressure for 15 minutes in order to precipitate the protein. After filtration the samples were diluted to a suitable volume for microbiological assay.

Total pantothenic acid was released from the animal tissues and foods by enzymatic digestion.[1] A quantity of sample estimated to contain 5 mcg. of pantothenic acid was weighed into a Maizel-Gerson reaction flask. A 2 ml. portion of glycine buffer (pH 8.5) was added to the sample and the

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[1]We are indebted to Dr. David Novelli for many helpful suggestions concerning the assay for pantothenic acid.

material was covered with a thin layer of toluene. Immediately before incubation two solutions of enzymes were added to the samples. Into each flask was added 0.1 ml. of a freshly prepared solution containing 100 mg. of intestinal phosphatase[1] dissolved in 10 ml. of distilled water. In addition a .05 ml. quantity of chicken liver enzyme preparation was added to the sample. The flasks were then placed in a water bath inside an incubator maintained at 37° C. for 4 hours. After incubation the pH of the samples was adjusted to 6.6 and the tissues were quantitatively transferred to 125 ml. erlenmeyer flasks, diluted to approximately 75 ml. with distilled water and autoclaved at 15 pounds pressure for 15 minutes to coagulate the protein. Filtration and dilution to a volume of 250 ml. completed the treatment.

The glycine buffer was prepared by adding 5 ml. of 1N sodium hydroxide to 95 ml. of a solution containing 75.05 gm. of glycine and 58.5 gm. of sodium chloride per liter. This buffer maintained the pH of the sample at approximately 8.5.

The chicken liver enzyme solution, which is considered to be very susceptible to losses of activity, was prepared in a cold room, and the tube containing the final solution was held in a beaker of ice water while being used. The preparation was made from acetone dried liver powder (Kaplan and Lipmann, 1948) which had been stored at 0° F. One gm.

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[1]Purchased from Armour and Company, Chicago, Illinois.

amounts of the liver powder were rubbed into 10 ml. of cold 0.02M sodium bicarbonate and the mixture was centrifuged at high speed for 15 minutes. The resulting red liquid was then decanted into a test tube which was held in ice water. This preparation was made up fresh before each series of assays.

Microbiological Assay Procedure

As pantothenic acid is present in urine in an uncombined state, no enzyme treatment was necessary. The samples were adjusted to a pH of 6.8 and diluted to a suitable volume. All analyses for pantothenic acid employed the microbiological technique. The synthetic media formulated by Landy and Dicken (1942) modified by the addition of 400 mcg. p-aminobenzoic acid per liter was used. Urine specimens for 16 rats (numbers 49187 to 49424) were assayed by means of Lactobacillus casei. All other assays were made with Lactobacillus arabinosus.

Lactic acid produced by the bacteria was titrated against 0.1N sodium hydroxide with brom thymol blue as the indicator.

The amount of pantothenic acid present in the enzymes used for liberation of bound pantothenic acid from tissues and the ration was determined, and concentrations were corrected accordingly.

RESULTS AND DISCUSSION

The Pantothenic Acid Requirement of the Rat for Reproduction Based on Analyses of the Developing Placentae and Fetuses

The deposition of pantothenic acid in fetal and placental tissues increased gradually throughout pregnancy and was most marked during those periods when growth was rapid (Figure 1). Placental tissue approached its maximum weight earlier in the gestation period than was found true for fetal tissue. By the 15th day of pregnancy the placentae averaged 5.2 gm. while at term the tissue had increased to somewhat over 7 gm. The average weight of the fetal mass was less than 3 gm. on the 15th day of pregnancy. This amount was doubled by the following day and development thereafter was unusually rapid to parturition. At birth the average weight per litter was 55 gm. In this study the average number of young per litter was 11.

Figure 2 and Table 1 give data on the quantity of pantothenic acid present in fetal and placental tissues at several stages of prenatal life. The placentae averaged 30 mcg. of pantothenic acid from the 16th day of pregnancy to term. Although growth was continued during this time, some reduction

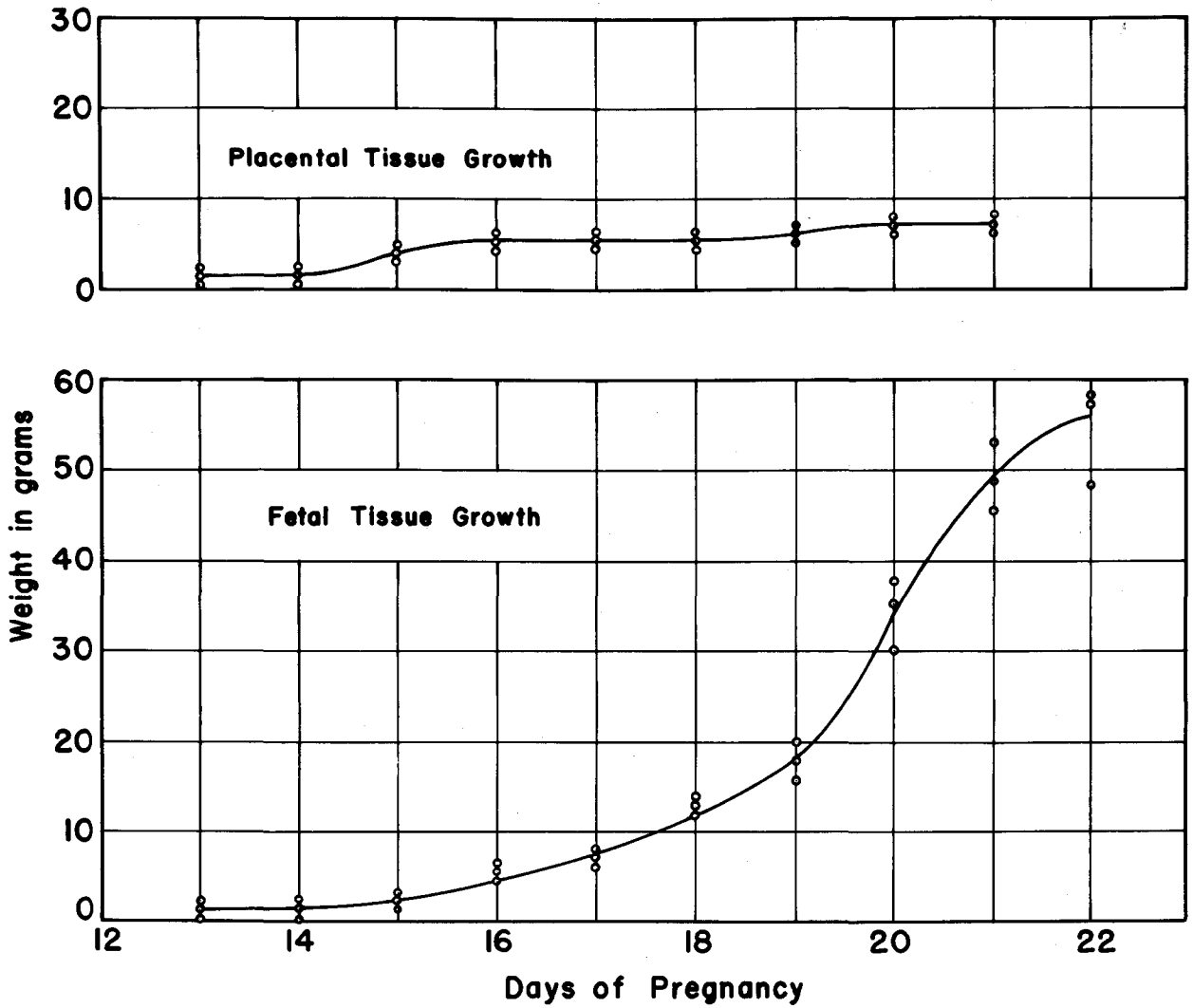

FIGURE 1. RATE OF GROWTH OF RAT TISSUES DURING PRENATAL LIFE.

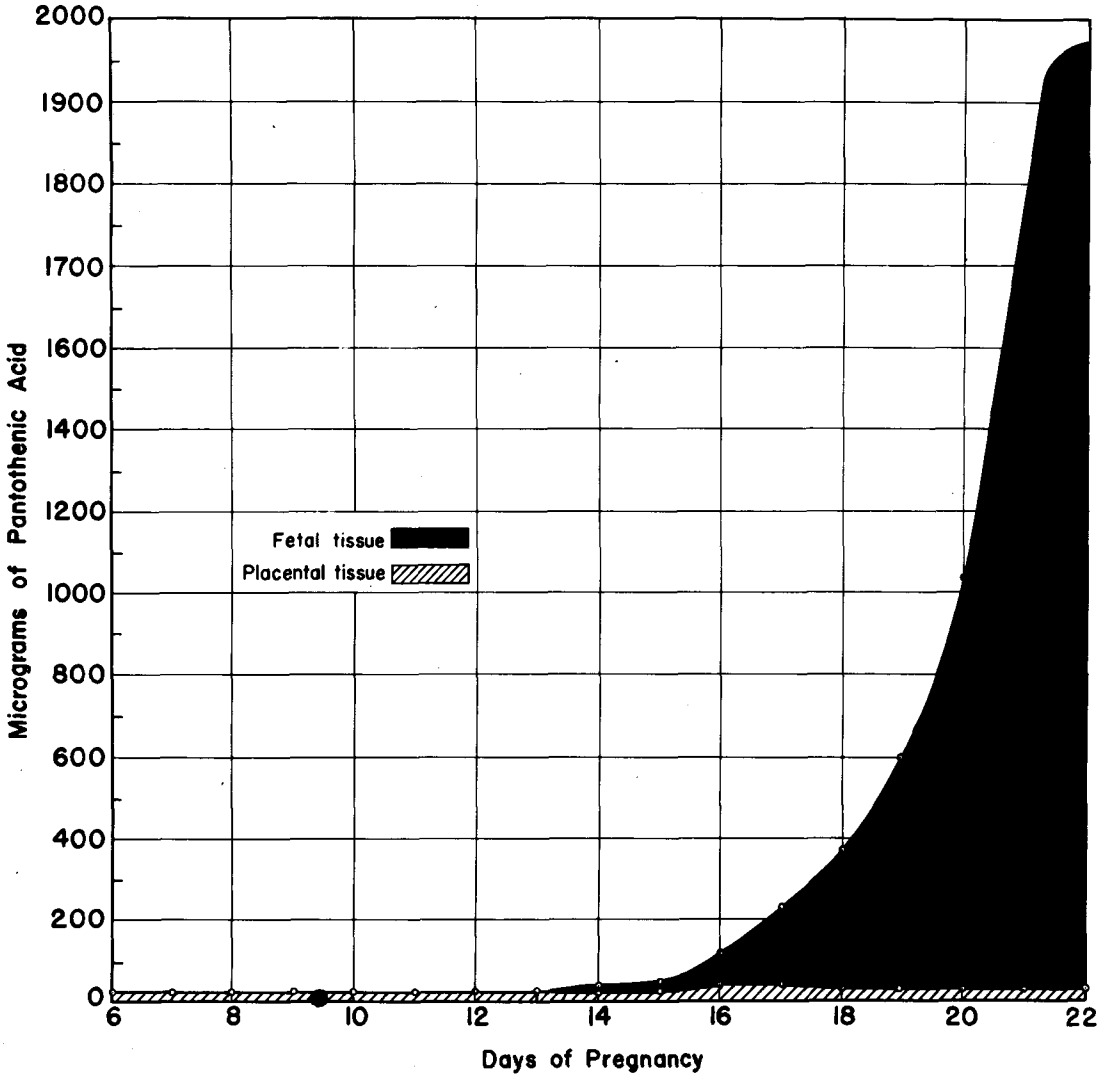

FIGURE 2. PANTOTHENIC ACID CONTENT OF PLACENTAL AND FETAL TISSUES AT VARIOUS STAGES OF PREGNANCY.

Table 1. Pantothenic Acid Content of Tissues of Stock Rats at Stages of Pregnancy.

Period of Pregnancy	Rat Number	Carcass		Liver		Uterus Contents		Number in Litter
		Per Gm.	Total	Per Gm.	Total	Per Gm.	Total	
		mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	
Non-pregnant	47715					1.7	2.0	
	47716					1.2	1.4	
	47910					0.8	0.9	
						1.2	1.4	
6 Days	47852	8.3	1111	53	426	0.5	0.4	
	47778	6.5	870	51	421	0.4	0.5	
	48646	7.5	988	58	519	3.6	5.8	
		7.4	990	54	455	1.5	2.2	
10 Days	48463	7.8	1037	66	557	5.9	16.0	12
	48464	7.6	1084	70	689	5.8	14.6	12
	48644	7.2	995	71	635	6.0	14.5	12
		7.5	1039	69	627	5.9	15.0	12
11 Days	47925	6.2	863	62	622	2.0	5.8	11
	47882	6.7	1047	66	661	6.6	25.0	15
	47937	7.6	1074	64	592	5.3	14.8	13
		6.8	995	64	625	4.6	15.2	13
12 Days	47911	7.6	1065	50	529	4.6	22.4	13
	47935	7.3	1040	55	513	3.8	19.9	14
	47853	6.2	1010	63	611	3.6	17.1	14
		7.0	1038	56	551	4.0	19.8	14

Table 1. (Continued)

Period of Pregnancy	Rat Number	Carcass		Liver		Placentae		Fetuses		Number in Litter
		Per Gm.	Total	Per Gm.	Total	Per Gm.	Total	Per Gm.	Total	
		mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	
13 Days	47828	7.0	988	71	678	1.4	3.0	3.5	4.3	10
	47896	7.2	1035	75	585	2.4	6.2	4.8	7.8	13
	47777	7.4	1288	67	635	5.0	8.0	11.5	12.6	12
		7.2	1104	71	633	2.9	5.7	6.6	8.2	12
14 Days	47936	7.4	1068	68	678	3.7	6.6	7.2	11.5	9
	47928	7.5	1089	66	554	2.4	3.9	13.4	18.8	12
	47909	7.4	1052	63	614	4.8	11.6	14.9	35.7	9
		7.4	1070	66	615	3.6	7.4	11.8	22.0	10
15 Days	47927	7.1	1019	68	670	4.9	17.5	9.2	23.8	11
	47913	7.9	1083	63	607	3.1	10.4	7.2	17.4	11
	48513	7.9	1070	60	595	6.2	23.1	7.3	23.9	12
		7.6	1057	64	624	4.7	17.0	7.9	21.7	11
16 Days	47796	7.4	1129	62	623	4.3	24.6	13.0	72.5	10
	47881	7.9	1234	72	710	5.8	25.7	17.9	93.1	12
	48505	7.4	1169	74	875	7.3	40.8	11.1	71.8	12
		7.6	1177	69	736	5.8	30.4	14.0	79.1	11
17 Days	47912	7.1	1007	58	518	6.9	30.9	25.2	166.0	10
	47854	7.3	1048	78	703	7.6	41.0	30.4	270.8	11
	48472	6.4	962	86	902	6.2	25.5	25.4	154.8	9
		6.9	1006	74	708	6.9	32.5	27.0	197.2	10

Table 1. (Continued)

Period of Pregnancy	Rat Number	Carcass		Liver		Placentae		Fetuses		Number in Litter
		Per Gm.	Total	Per Gm.	Total	Per Gm.	Total	Per Gm.	Total	
		mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	mcg.	
18 Days	47827	6.9	936	65	561	4.5	25.7	21.4	299	10
	47851	7.0	1072	64	644	3.8	20.8	25.9	365	10
	47903	6.9	990	75	644	6.3	30.8	27.8	345	10
		6.9	999	68	616	4.9	25.8	25.0	336	10
19 Days	47798	7.1	1085	71	689	4.7	35.7	29.1	535	11
	47902	6.9	1019	75	701	4.3	26.0	31.1	635	9
	47895	7.2	1072	60	588	4.1	23.8	34.5	552	9
		7.1	1059	69	659	4.4	28.5	31.6	574	10
20 Days	47750	6.2	1000	77	789	4.2	26.7	23.3	828	10
	47722	6.2	976	61	581	3.5	27.8	29.1	1079	13
	48640	6.1	896	82	880	6.6	41.4	35.8	1078	11
		6.2	957	73	750	4.8	32.0	29.4	995	11
21 Days	47717	7.1	1048	68	594	4.0	26.1	32.8	1506	10
	47749	6.4	975	69	641	4.2	31.8	35.1	1699	11
	47799	6.5	857	62	535	3.7	29.6	32.7	1737	10
		6.7	960	66	590	4.0	29.2	33.5	1647	10
Full Term	47718	6.9	966	67	597	-	-	42.9	2090	10
	47721	7.3	1035	65	637	-	-	32.8	1908	12
	47751	6.8	1009	61	606	-	-	31.9	1844	11
		7.0	1003	64	613	-	-	35.9	1947	11

in the concentration of pantothenic acid occurred in placental tissue.

The deposition of the vitamin in fetal tissue took place at a very accelerated rate during the latter portion of pregnancy. The quantity of vitamin increased from an average of 2 mcg. on the 6th day of pregnancy to 1947 mcg. at term. While rapid increase in fetal mass accounted to some extent for the very high amounts of pantothenic acid present in the total fetal tissue, there was a very noticeable increase in the concentration of pantothenic acid per unit of tissue during prenatal development. This concentration was approximately 6 fold.

Analyses of fetal tissues at daily intervals during reproduction have provided a fairly accurate means of estimating the quantity of pantothenic acid being deposited at any portion of the gestation period. These values are given in Table 2. The maximum daily deposition of the vitamin, i.e., 653 mcg. occurred during the 21st day of pregnancy, although the amounts of pantothenic acid transferred to the young during the 18th and 19th days were also relatively large.

If the newborn rats produced by females receiving the modified Steenbock stock ration are in an optimum state of nutrition with regards to their pantothenic acid stores, approximately 650 mcg. of this vitamin might be considered the minimum daily requirement for reproduction in rats during

the latter part of pregnancy. Figure 3 presents estimations of the daily needs of the vitamin which are in excess of those of the non-pregnant adult rat.

It was calculated that the experimental animals of this group ingested between 500 and 600 mcg. of pantothenic acid

Table 2. Average Daily Increment in Pantothenic Acid Observed in Fetal and Placental Tissues during Pregnancy.

Period of Pregnancy	Pantothenic Acid Transferred to the Fetuses	Pantothenic Acid Added to the Placentae
Day	mcg.	mcg.
14	14	1.7
15	0	9.6
16	57	13.4
17	118	2.1
18	139	-6.7
19	238	2.7
20	421	3.5
21	653	-2.8
Full term	300	0

daily. This quantity of vitamin was furnished from 10 to 12 gm. of the stock ration plus a supplement of beef or carrot fed on alternate days. The stock ration was found to provide 43.9 mcg. of pantothenic acid per gm. of feed, raw beef contained 5.6 mcg. per gm. and carrots contributed 5.0 mcg. per gm. This daily intake of pantothenic acid appears ample for the early portion of pregnancy but was not

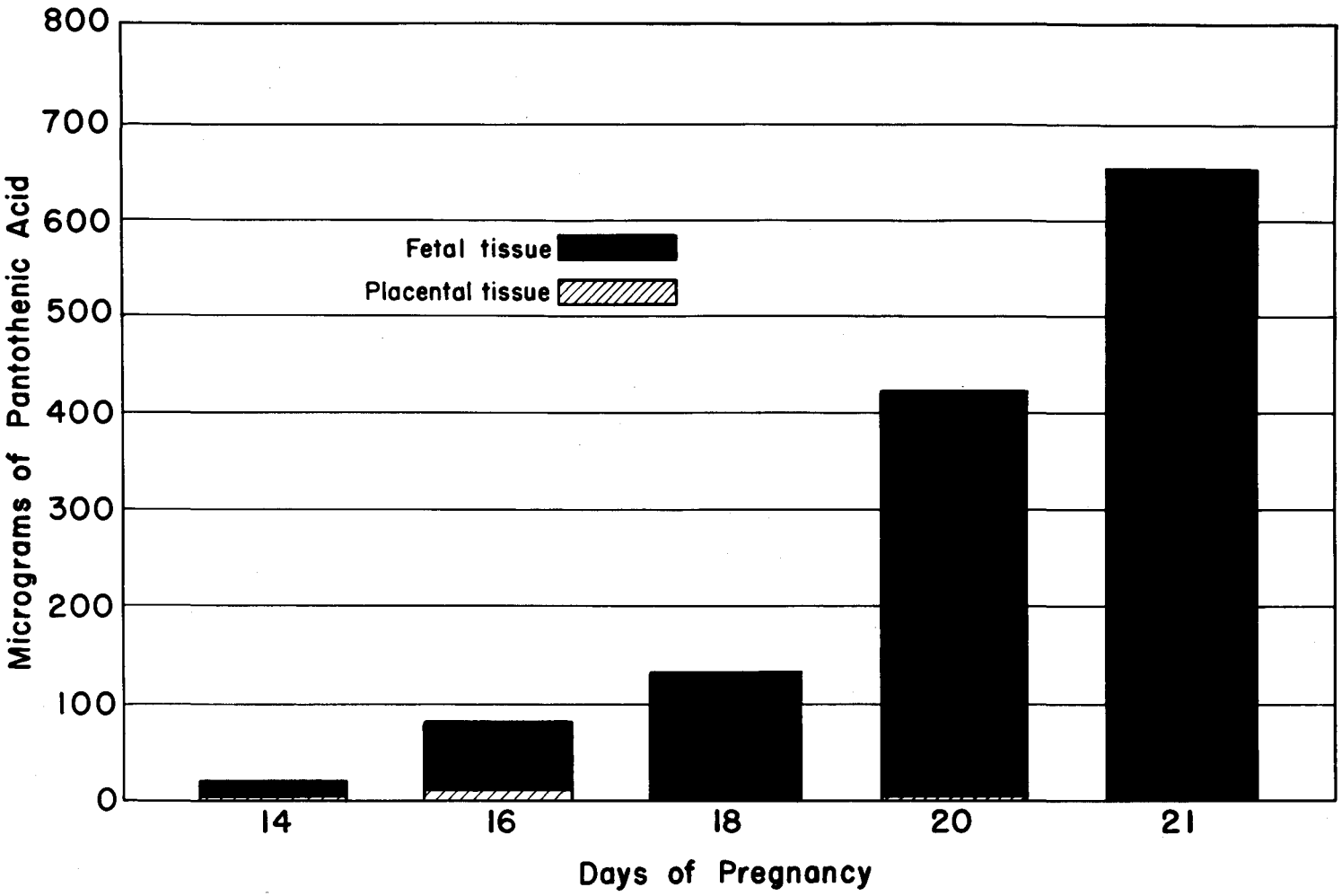

FIGURE 3. DAILY MINIMUM PANTOTHENIC ACID REQUIREMENTS FOR NORMAL REPRODUCTION IN RATS BASED ON TISSUE ANALYSIS.

equal to the amount of vitamin deposited in fetal tissues during the 21st day of the gestation period.

Since the intake and apparent demand for pantothenic acid were very similar, considerable interest was taken in determining the maternal stores of this factor. It will be observed from Table 1 that there was no consistent trend toward lower or higher concentrations of pantothenic acid in the maternal liver or carcass as pregnancy progressed. The total quantity of pantothenic acid present in carcass tissue (averaged for each group of 3 animals) varied from 957 to 1177 mcg. These data illustrate a considerable degree of uniformity in the body stores of pantothenic acid. This is especially true in view of the difficulties encountered in preparing and sampling this tissue. The quantity of pantothenic acid present in the liver varied more widely, with averages of a given group of animals ranging from 455 to 750 mcg. Again the differences followed no definite order.

Little if any evidence of depletion of maternal stores was detectable by the technique used in the present study. Likewise, it was apparent that the rat did not store any quantity of the vitamin during early phases of the reproductive cycle which might enable it to transfer pantothenic acid to the young at a more critical stage of reproduction.

It was intriguing to observe that the female rat was able to transfer a quantity of pantothenic acid, beyond that of its entire body stores to the developing young within a

period of 7 or 8 days. These findings imply an unusually high requirement for pantothenic acid during pregnancy. Other approaches to the quantitative aspects of this requirement will follow.

The Excretion of Pantothenic Acid and Tissue Stores of Pregnant and Non-pregnant Females Consuming the Stock Ration With or Without Supplements of Calcium Pantothenate

Observations on reproductive performance

The reproductive performance of rats ingesting the stock ration without supplementation is shown in Table 3. Nine females gave birth to an average of 8.2 young with an average birth weight of 5.1 gm. The young born during this experiment were of uniform size and appeared to be healthy. Autopsy showed that livers of the adult rats were of normal color, but examination of the uteruses revealed that resorptions had occurred in four animals.

Addition of calcium pantothenate to the ration at levels of .002 and .005 per cent during the last 6 days of pregnancy was followed by reproductive success of the same order. This was also true of the two animals fed the ration supplemented with .005 per cent calcium pantothenate throughout pregnancy. Table 4 includes data of rats fed the ration fortified with the vitamin. The average number of rats per litter was 9.3 with an average birth weight of 4.9 gm. Although the number

Table 3. Reproductive Performance of Rats Fed the Stock Ration During Pregnancy.

Rat No.	No. Young	No. Resorptions	Wt. of Litter	Average Wt. per rat
			gm.	gm.
49202	9	0	43.6	4.8
49323	12	0	56.0	4.7
49239	6	0	32.8	5.5
50259	8	3	41.7	5.2
50900	10	1	51.0	5.1
50915	10	1	51.3	5.1
50946	4	0	21.3	5.3
50947	9	0	48.3	5.4
50948	6	1	34.4	5.7
Average	8.2	0.7	42.3	5.1

Table 4. Reproductive Performance of Rats Fed the Stock Ration Supplemented with Calcium Pantothenate

Rat No.	Per Cent Ca Pant. in Ration	Day Supplement added	No. Young	No. Resorptions	Wt. of Litter	Av. Wt. per Rat
					gm.	gm.
50297	.002	16	11	0	56.6	5.1
50298	.002	16	5	1	27.5	5.5
50892	.005	16	10	1	44.1	4.4
50894	.005	16	8	2	41.8	5.2
50914	.005	16	10	1	47.0	4.7
50917	.005	16	9	3	42.4	4.7
50891	.005	1	9	1	42.6	4.7
50902	.005	1	12	0	55.9	4.7
Average			9.3	1.1	44.7	4.9

of young per litter was higher in the group fed the enriched ration, no real improvement in reproductive performance could be attributed to the diet. The incidence of resorption was higher among these animals than among the controls.

From the general response of the two groups of animals it did not appear that there was any beneficial effect of increasing the quantity of pantothenic acid beyond that present in the customary stock diet. In either case reproductive performance was good.

Excretion of pantothenic acid in the urine

The daily ingestions and excretions of pantothenic acid for rats approximately six weeks old are given in Table 5. The intake of pantothenic acid was calculated from assays of the various food items, -the stock ration, beef, and carrots. (Data concerning the pantothenic acid content of these foods for the four series of animals included in this investigation are given in the appendix.)

The amount of vitamin ingested fluctuated somewhat during this time depending upon the quantity of stock diet selected. Food intake varied from 6.8 to 11.0 gm. per day with an average of 9.1 gm. for all animals during the six collection periods. There appeared to be no increase in the amount of food consumed during the metabolism experiments when the animals were 46 to 70 days old. The average

Table 5. Daily Intake and Excretion of Pantothenic Acid of Rats 6 Weeks of Age.

Rat Number		Days 4	8	12	16	20	24
49187	Intake (mcg.)	502	544	544	671	418	586
	Excretion(mcg.)	168	182	152	172	171	184
	% Excreted	33	33	28	26	41	31
49188	Intake (mcg.)	530	530	516	502	558	474
	Excretion(mcg.)	164	179	166	196	157	157
	% Excreted	31	34	32	39	28	33
49189	Intake (mcg.)	502	516	572	572	601	572
	Excretion(mcg.)	175	180	167	205	209	209
	% Excreted	35	35	29	36	35	37
49190	Intake (mcg.)	558	488	530	544	544	516
	Excretion(mcg.)	178	183	182	180	184	178
	% Excreted	32	38	35	33	34	35
49201	Intake (mcg.)	474	516	530	544	572	558
	Excretion(mcg.)	126	154	171	166	208	200
	% Excreted	27	30	32	31	36	36
49202	Intake (mcg.)	446	586	544	558	558	629
	Excretion(mcg.)	164	130	133	208	221	217
	% Excreted	37	22	24	37	39	34
49203	Intake (mcg.)	516	572	586	629	615	601
	Excretion(mcg.)	171	176	192	184	252	273
	% Excreted	33	31	33	29	40	45
49204	Intake (mcg.)	544	629	601	615	601	558
	Excretion(mcg.)	181	176	159	197	196	220
	% Excreted	33	28	26	32	33	39

Table 5. (Continued)

Rat Number		Days 4	8	12	16	20	24
49231	Intake (mcg.)	516	502	572	586	502	601
	Excretion(mcg.)	158	153	170	210	245	204
	% Excreted	31	30	30	36	49	34
49232	Intake (mcg.)	502	516	558	516	572	572
	Excretion(mcg.)	152	145	171	196	190	182
	% Excreted	30	28	31	38	33	32
49233	Intake (mcg.)	502	643	544	558	530	544
	Excretion(mcg.)	138	142	167	192	180	171
	% Excreted	27	22	31	34	34	31
49234	Intake (mcg.)	530	530	657	544	544	615
	Excretion(mcg.)	164	144	203	212	246	163
	% Excreted	31	27	31	39	45	27
49239	Intake (mcg.)	516	558	572	530	586	558
	Excretion(mcg.)	165	121	176	255	199	207
	% Excreted	32	22	31	48	34	37
49240	Intake (mcg.)	488	516	530	558	558	502
	Excretion(mcg.)	167	144	181	210	191	209
	% Excreted	34	28	34	38	34	42
49241	Intake (mcg.)	516	530	516	530	572	530
	Excretion(mcg.)	157	160	183	187	241	269
	% Excreted	30	30	35	35	42	51
49242	Intake (mcg.)	530	629	601	601	629	643
	Excretion(mcg.)	178	141	174	211	219	215
	% Excreted	34	22	35	35	35	33

pantothenic acid intake was 552 mcg. per day. Of this amount, approximately one-third, or an average of 183 mcg. could be accounted for in the urine.

A portion of these animals were continued on metabolism experiments while litter mates were mated. These animals, now approximately 10 weeks old, were kept in metabolism cages for an additional 22 days, an interval comparable to the gestation period. Data on the non-pregnant animals are given in Table 6. Food intakes were approximately the same, an average of 9.0 gm. per day, and pantothenic acid excretion appeared to be very similar to that of younger animals. Because of this uniformity in excretion it may be assumed that the excretion values of pregnant animals were not complicated by changes brought about by aging of the rats. Examples of these balance studies are shown in Figure 4 which illustrates the intake and excretion of pantothenic acid from the time the rats were 46 days of age through the age of 96 days. A very short interruption occurred when certain of the animals were mated. (Graphs showing the pantothenic acid intake and excretion of the remainder of the animals on metabolism experiments are included in the appendix).

The most striking characteristic of the pantothenic acid excretion of pregnant animals ingesting the stock ration was the sharp decline during the last 6 days before parturition. Table 7 gives values of both intake and excretion during the

Table 6. Daily Intake and Excretion of Pantothenic Acid by Non-pregnant Rats 10 Weeks of Age.

Rat Number		Day of Pregnancy 4	6	10	12	14	16	18	20	22
49188	Intake (mcg.)	629	699	713	601	488	601	488	572	-
	Excretion(mcg.)	173	233	207	272	200	227	177	198	-
	% Excreted	28	33	29	45	41	38	36	35	-
49190	Intake (mcg.)	601	601	601	488	516	629	432	629	-
	Excretion(mcg.)	182	239	161	258	146	213	184	164	-
	% Excreted	30	40	27	53	28	34	43	23	-
49203	Intake (mcg.)	586	601	432	601	601	488	685	460	713
	Excretion(mcg.)	201	177	186	111	226	138	193	216	143
	% Excreted	34	29	43	18	38	28	28	47	20
49233	Intake (mcg.)	572	572	601	516	601	601	572	601	488
	Excretion(mcg.)	220	204	220	258	194	208	177	205	270
	% Excreted	38	36	37	50	32	35	31	34	55
49240	Intake (mcg.)	488	460	544	404	657	460	516	601	657
	Excretion(mcg.)	210	206	142	214	152	241	156	207	239
	% Excreted	43	45	26	53	23	52	30	34	36
50893	Intake (mcg.)	429	442	389	523	469	469	469	442	496
	Excretion(mcg.)	134	134	187	124	195	138	135	200	142
	% Excreted	31	30	48	24	24	29	29	45	29
50898	Intake (mcg.)	375	483	496	335	603	496	496	523	389
	Excretion(mcg.)	102	120	119	151	103	229	164	249	197
	% Excreted	27	25	24	45	17	46	33	48	51
50934	Intake (mcg.)	550	577	577	550	442	577	469	603	469
	Excretion(mcg.)	170	180	192	222	144	147	249	129	251
	% Excreted	31	31	33	40	33	25	53	21	54

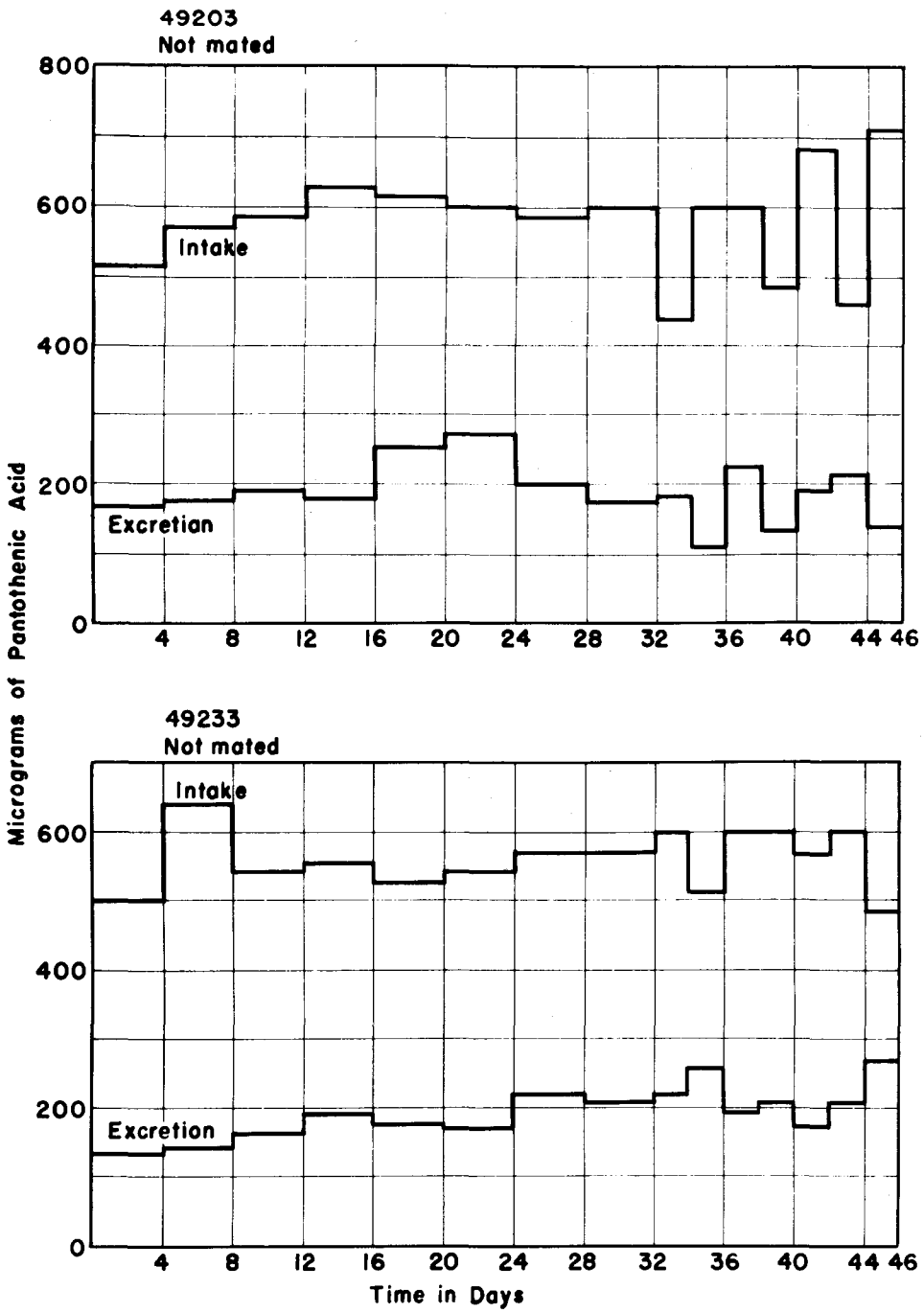

FIGURE 4. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY 6 WEEKS OLD RATS #49203 AND #49233.

Table 7. Daily Intake and Excretion of Pantothenic Acid by Pregnant Rats 10 Weeks of Age.

Rat Number		Day of Pregnancy 4	8	10	12	14	16	18	20	22
49202	Intake (mcg.)	657	699	601	601	601	825	769	685	-
	Excretion(mcg.)	197	222	212	146	176	151	108	36	-
	% Excreted	30	32	35	24	29	18	14	5	-
49232	Intake (mcg.)	629	643	685	769	825	629	713	741	827
	Excretion(mcg.)	176	172	166	184	180	181	122	63	25
	% Excreted	28	27	24	24	22	29	17	9	3
49239	Intake (mcg.)	657	699	685	713	769	769	741	741	771
	Excretion(mcg.)	212	232	234	259	239	225	168	102	58
	% Excreted	32	33	34	36	31	29	23	13	8
50259	Intake (mcg.)	469	588	601	708	681	575	734	787	654
	Excretion(mcg.)	125	136	213	141	191	222	81	48	23
	% Excreted	27	23	35	20	28	39	11	6	4
50900	Intake (mcg.)	469	509	550	577	577	684	657	603	684
	Excretion(mcg.)	175	190	162	169	130	84	85	42	24
	% Excreted	37	37	29	29	23	12	13	7	4
50915	Intake (mcg.)	483	577	550	577	603	684	550	711	657
	Excretion(mcg.)	152	188	205	153	165	155	109	24	10
	% Excreted	31	33	37	27	27	23	20	3	2
50946	Intake (mcg.)	496	536	630	603	523	684	684	657	657
	Excretion(mcg.)	201	219	221	185	216	239	191	108	62
	% Excreted	41	41	35	31	41	35	28	16	9

Table 7. (Continued)

Rat Number		Day of Pregnancy 4	8	10	12	14	16	18	20	22
50947	Intake (mcg.)	509	536	603	577	711	630	711	738	630
	Excretion(mcg.)	162	233	238	137	228	172	135	53	25
	% Excreted	32	43	39	24	32	27	19	7	4
50948	Intake (mcg.)	536	523	630	711	603	738	791	711	791
	Excretion(mcg.)	248	178	218	163	205	249	188	82	49
	% Excreted	46	34	35	23	34	34	24	12	6

gestation period. Figures 5 and 6 show graphically the changes observed in four rats. Excretions reached low levels despite the fact that the intake of pantothenic acid was as high or slightly higher than that of the virgin animals of the same age. The excretion of the vitamin was most markedly decreased in animals which produced large litters.

Figure 5 shows that the excretion of pantothenic acid after mating was essentially the same as that for the preceding 12 days, until the latter part of pregnancy. The decline in excretion of this vitamin by rat # 49202 occurred on the 10th day of pregnancy while in the case of rat # 49232 and most other animals, the decline was not observed until the 16th day. Rat # 50915 excreted only 10 mcg. of pantothenic acid per day during the last 2 days of pregnancy and produced a litter of 10 young weighing 51.3 gm. The smallest litter produced on the unsupplemented stock diet included 4 young weighing 21.3 gm., born to rat # 50946. This female excreted 62 mcg. of pantothenic acid two days before delivery.

Although the modified Steenbock ration was believed to support good reproduction among stock animals, the low excretion of pantothenic acid toward the end of the gestation period suggested that a higher intake of the vitamin might have advantages. Supplementation of the ration with calcium pantothenate during the last 6 days of pregnancy brought about only slight increases in the amount of pantothenic acid

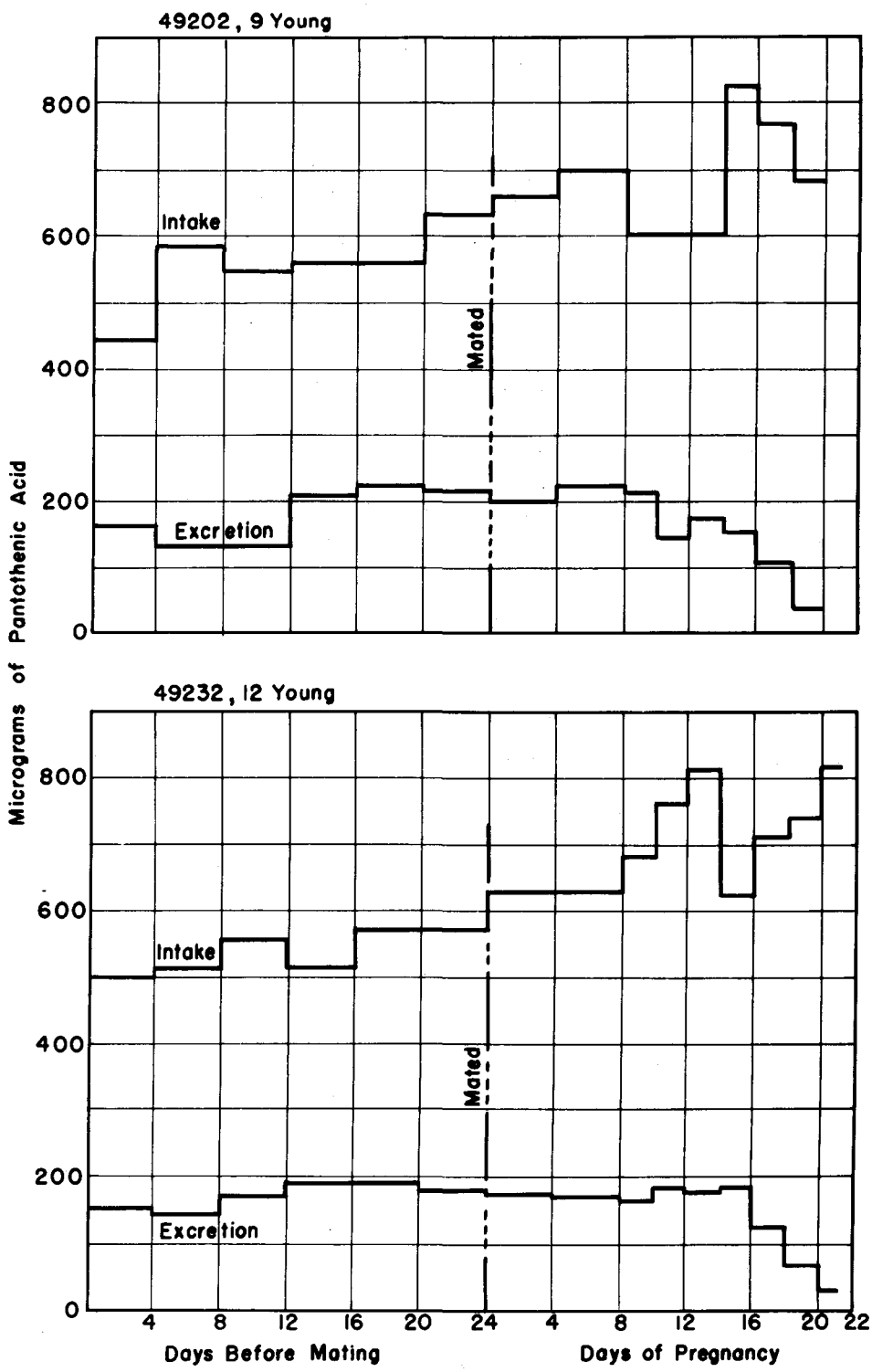

FIGURE 5. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RATS #49202 AND #49232.

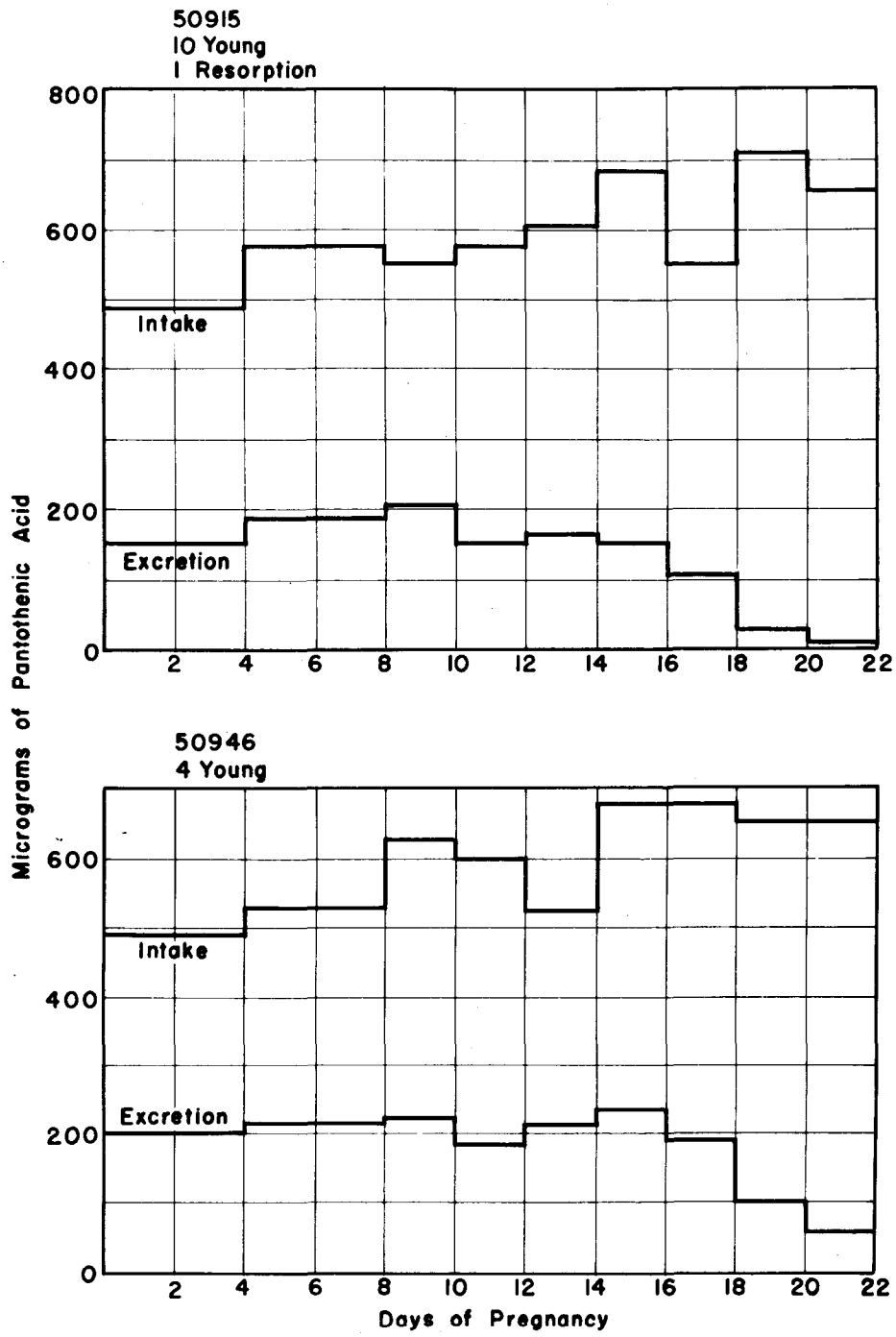

FIGURE 6. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RATS #50915 AND #50946.

excreted in the urine. These results are illustrated in Table 8 and Figures 7, 8, and 9. Again animals which were producing the largest litters excreted the least pantothenic acid.

Addition of extra pantothenic acid throughout the entire gestation period caused an increased amount of the vitamin to be excreted during the earlier part of pregnancy, however, the typical lowered excretion during the last 6 days was still apparent. Table 9 and Figure 10 give information concerning the intake and excretion of pantothenic acid when the stock ration was supplemented with .005 per cent calcium pantothenate from the initiation of pregnancy.

Several questions have arisen in attempting to interpret the results of the metabolism experiments. First, it was unexpected to find that there was no rise in urinary excretion of pantothenic acid during the period from 46 to 96 days in the life of the healthy young female rat. This interval of time for the rat included a period of growth which extended into the early adult life of the animal. Since several investigators have suggested that the requirement for this vitamin decreases in the older animal, it was thought that some change in needs would be reflected in the urinary excretion of pantothenic acid.

The excretion by adult rats of approximately 33 per cent of an intake roughly twenty times the suggested requirement

Table 8. Daily Intake and Excretion of Pantothenic Acid of Rats Ingesting the Stock Ration Supplemented with Calcium Pantothenate on the 16th Day of Pregnancy.

		Day of Pregnancy								
		4	8	10	12	14	16	18	20	22
.002% Calcium Pantothenate										
50297	Intake (mcg.)	508	615	522	628	734	708	884	921	848
	Excretion(mcg.)	154	175	249	202	138	207	164	76	23
	% Excreted	30	29	48	32	19	29	19	8	3
50298	Intake (mcg.)	535	601	575	681	761	708	1031	1031	921
	Excretion(mcg.)	149	164	244	191	207	163	144	144	167
	% Excreted	28	27	42	28	27	23	14	14	18
.005% Calcium Pantothenate										
50892	Intake (mcg.)	483	536	550	550	684	523	1025	1077	1025
	Excretion(mcg.)	116	161	153	178	184	150	341	167	73
	% Excreted	24	30	28	32	27	29	33	16	7
50894	Intake (mcg.)	496	577	577	657	657	684	1077	1232	1025
	Excretion(mcg.)	146	200	172	163	163	116	261	158	97
	% Excreted	29	35	30	25	25	17	24	13	10
50914	Intake (mcg.)	509	577	550	630	630	630	1232	1180	1193
	Excretion(mcg.)	139	144	133	133	147	105	158	112	41
	% Excreted	27	25	24	21	23	17	13	10	3
50917	Intake (mcg.)	536	590	550	684	684	550	1388	1284	973
	Excretion(mcg.)	78	152	173	133	209	156	230	134	72
	% Excreted	15	26	32	19	31	28	17	10	7

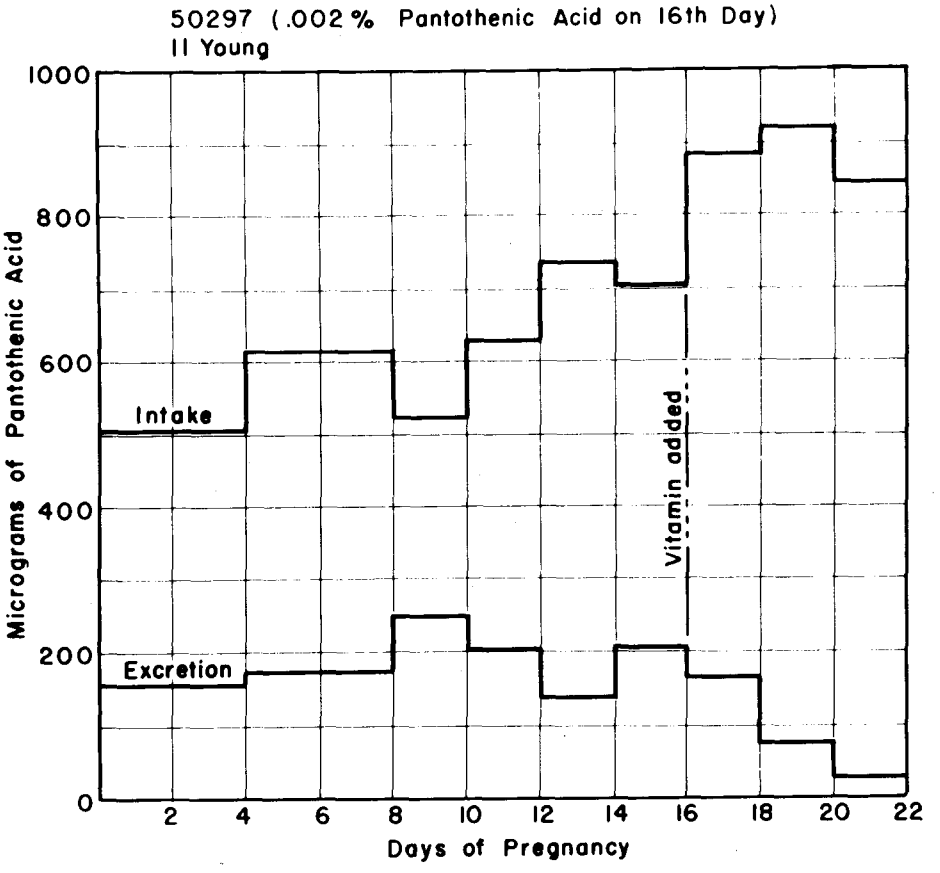

FIGURE 7. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50297 CONSUMING THE RATION SUPPLEMENTED WITH .002 PER CENT PANTOTHENIC ACID ON THE 16TH DAY.

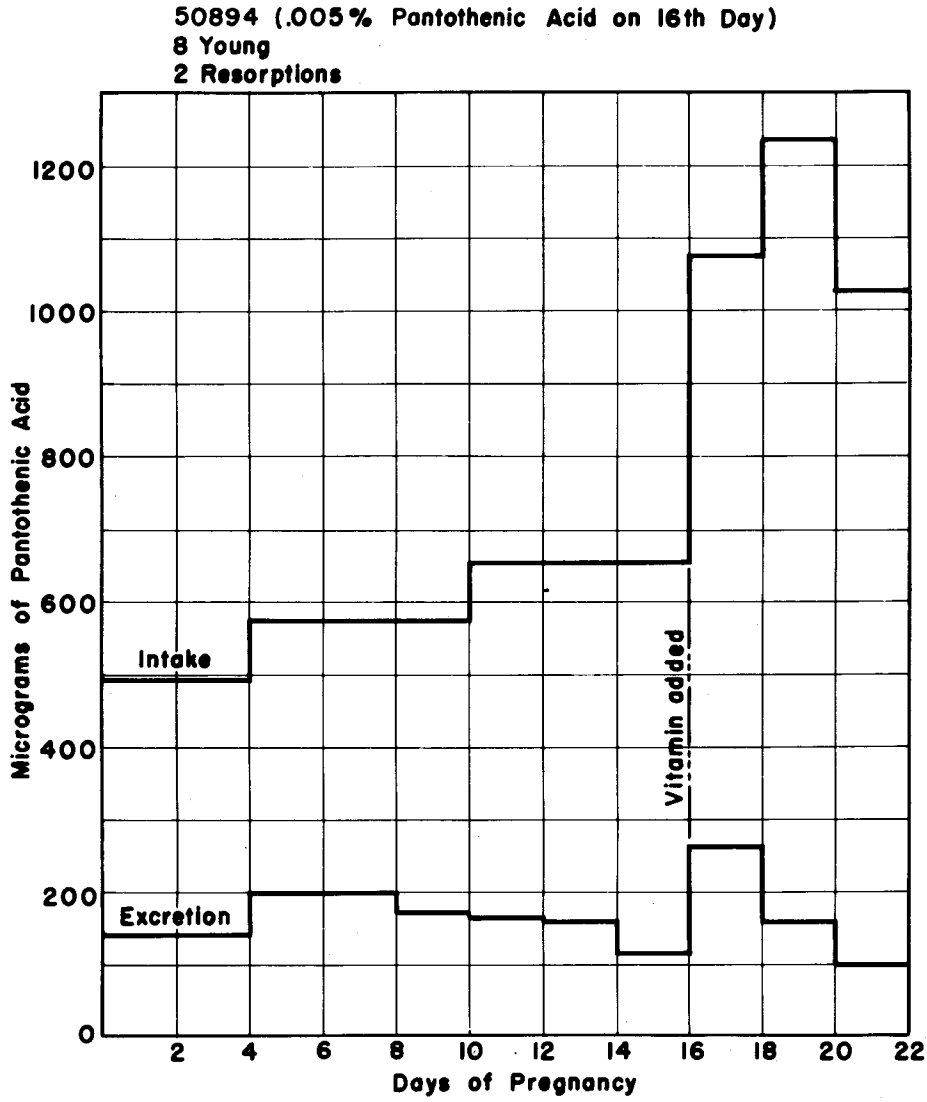

FIGURE 8. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50894 CONSUMING THE RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID ON THE 16TH DAY OF PREGNANCY.

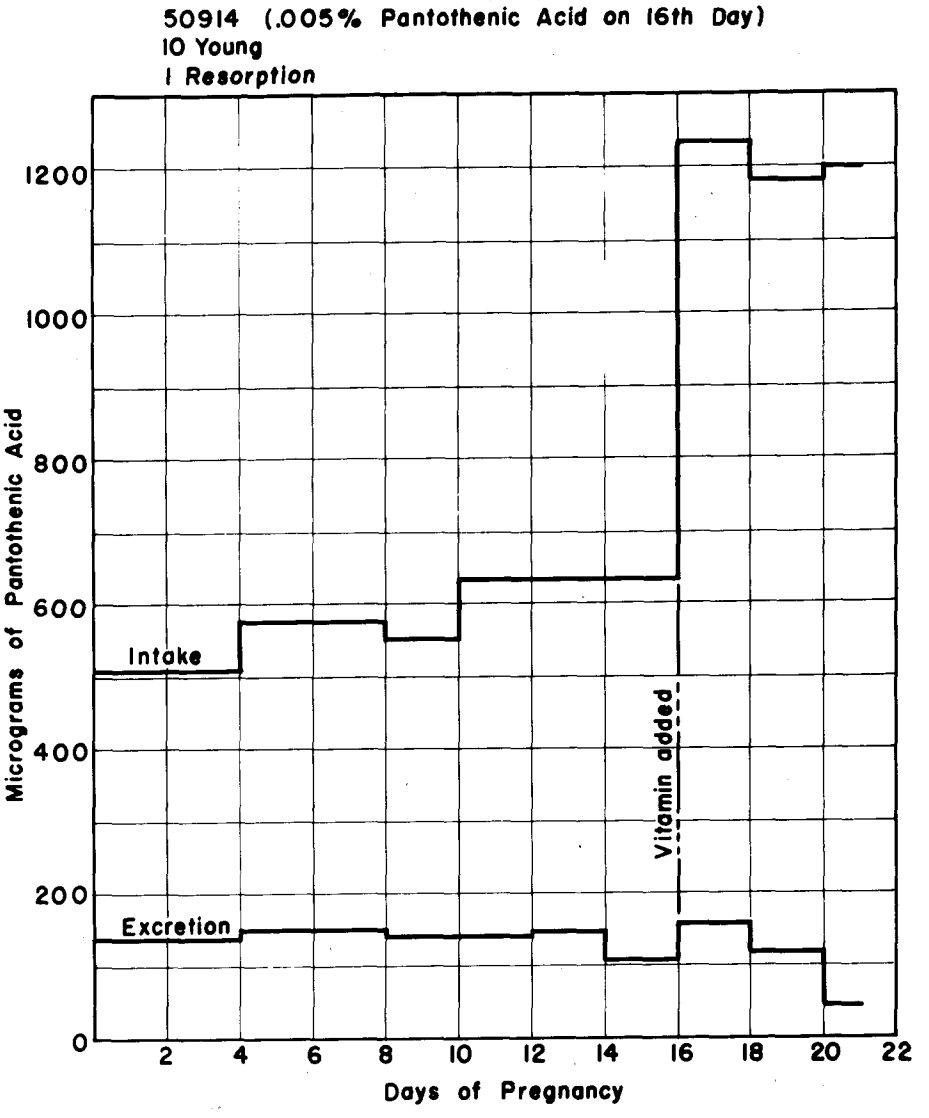

FIGURE 9. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50914 CONSUMING THE STOCK RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID ON THE 16TH DAY OF PREGNANCY.

Table 9. Daily Intake and Excretion of Pantothenic Acid of Rats Ingesting the Stock Ration Supplemented with .005 Per Cent Calcium Pantothenate Throughout Pregnancy.

Rat Number		Day of Pregnancy 4	8	10	12	14	16	18	20	22
50891	Intake (mcg.)	846	947	973	1077	1180	1077	869	1128	1077
	Excretion(mcg.)	388	450	450	575	653	618	314	163	65
	% Excreted	46	48	47	53	61	57	36	15	6
50902	Intake (mcg.)	1051	947	1077	1077	869	1232	1180	1491	1128
	Excretion(mcg.)	406	488	441	428	463	372	328	172	60
	% Excreted	39	52	41	40	53	30	28	12	5

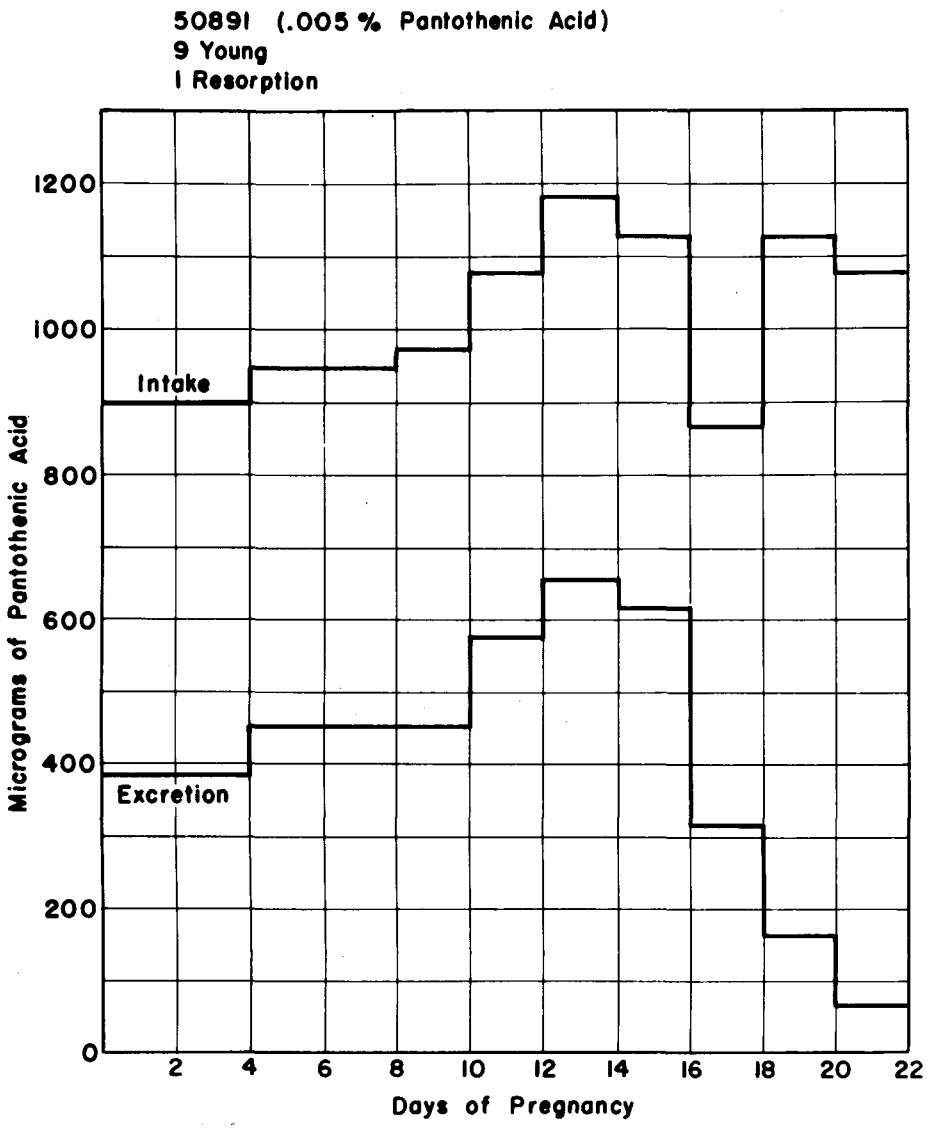

FIGURE 10. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50891 CONSUMING THE RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID FROM THE ONSET OF PREGNANCY.

for pantothenic acid allows speculation as to the fate of the remainder of the vitamin. Unna and Richards estimated that the needs of the adult rat would be met with an intake of 25 mcg. per day, while experimental animals of this study ingested foods which supplied approximately 500 mcg. of the vitamin daily. Although it is assumed that a fraction of the pantothenic acid present in the mixture of foods might escape absorption, a large portion of the dietary intake is not accounted for in the metabolism experiments.

When the observation was first made that there was a definite reduction in the quantity of pantothenic acid excreted in the urine of pregnant animals during the last 6 days of pregnancy, it was assumed that this drop meant that the diet no longer provided a surplus of the vitamin due to the rapid growth of the fetuses at the end of pregnancy. Possibly this idea is correct. Rather good agreement exists between the number of young or fetal mass being produced and the actual amount of pantothenic acid present in the urine at the end of pregnancy. Data from the analyses of fetal tissues during the last quarter of gestation would also lend some support to the idea that the stock ration did not provide any great surplus of pantothenic acid.

The addition of extra pantothenic acid either at the beginning of pregnancy or on the 16th day failed to prevent the drop in excretion of this vitamin during the last few

days of reproduction. The amount of vitamin supplied, approximately 200 or 500 mcg. of calcium pantothenate per day should have been ample to reveal whether the stock ration was not quite adequate for the last days of pregnancy. The fact that the urinary excretion of the vitamin followed the same pattern regardless of whether the ration was supplemented with pantothenic acid or fed as such must mean that either excessive amounts of the vitamin were being transferred to the young or that poorer absorption of the vitamin occurred during the latter portion of pregnancy. Some evidence for the first suggestion has been found from additional data to follow.

The Concentration of Pantothenic Acid in Tissues of Rats Fed the Stock Ration and the Effect of Adding Calcium Pantothenate

The concentration of pantothenic acid per gm. of carcass or hepatic tissue showed a gradual decline over the age period studied. These values are given in Tables 10 and 11. When virgin animals were sacrificed at 6, 10, or 13 weeks of age the carcasses contained on an average 10.1, 9.4 and 7.6 mcg. of pantothenic acid per gm. of fresh tissue respectively. The livers of the same animals contained 126, 93, and 82 mcg. of the vitamin per gm. Since animals in this study were mated when they were approximately 10 weeks old, this downward trend in tissue stores per unit of weight was

still in effect. While the number of animals is limited it should be mentioned that there appeared to be greater variability among females approximately 13 weeks of age than was true for younger animals. This variability cannot be explained.

Table 10. Pantothenic Acid Content of Tissues of Young Female Rats Fed the Stock Ration.

Rat Number	Age in Days	Carcass		Liver	
		Per Gm.	Total	Per Gm.	Total
		mcg.	mcg.	mcg.	mcg.
49279	43	9.3	542	132	632
49280	43	11.1	635	101	506
49287	40	10.2	626	129	516
49288	42	9.8	590	140	754
Average		10.1	598	126	602
49191	70	9.7	916	87	486
49205	70	8.9	814	95	595
49235	71	9.8	962	107	794
49243	71	9.1	875	81	662
Average		9.4	892	93	634

It will be recalled that these animals excreted very nearly the same amount of pantothenic acid in the urine from the 6th through the 13th week of life. Since the rats did not excrete additional pantothenic acid as aging occurred and the concentration of their tissues decreased their dietary needs for this vitamin were appreciably decreased.

The average concentration of the vitamin per gm. of fresh tissue was actually higher in livers of animals which had produced litters. The concentration per gm. of hepatic tissue for virgin rats averaged 82 mcg. as compared with 91 mcg. for animals postpartum. This small difference in concentration

Table 11. Bound and Free Pantothenic Acid in Tissues of Virgin Female Rats Fed the Stock Ration.

Rat Number	Age in Days	Carcass		Liver		Free
		Per Gm.	Total	Per Gm.	Total	
		mcg.	mcg.	mcg.	mcg.	% of Total
49188	91	7.7	846	61	411	2.6
49190	91	7.8	821	93	607	2.7
49203	93	6.1	684	82	612	1.6
49233	92	6.0	646	92	572	2.9
49240	92	8.8	886	62	409	1.9
50893	98	6.7	734	90	520	2.6
50898	94	9.6	1075	96	630	2.2
50934	90	8.4	963	82	640	-
Average		7.6	832	82	550	2.4

probably has little significance as individual values in both groups covered a wide range. The data show, however, that the maternal stores of the vitamin were not depleted when females were maintained on the stock ration during the reproductive cycle.

Free pantothenic acid was assayed in livers to observe any change in the distribution of bound and free forms of the

vitamin as the result of pregnancy or dietary supplementation. All liver values for free pantothenic acid were low, regardless of whether the female had just produced a litter or the diet had been supplemented with calcium pantothenate. The amount of free vitamin present in this organ never exceeded 3 mcg. per gm. This quantity represented between 3 and 4 per cent of the total pantothenic acid. These values were very similar to those observed by Novelli, Kaplan and Lipmann (1949) who have suggested that pantothenic acid is present in animal tissues mainly in bound form, probably as coenzyme A.

The amount of pantothenic acid present in the entire bodies of newborn rats born to females maintained on the usual stock ration averaged 45 mcg. per gm. of fresh tissue or 1856 mcg. per litter. These figures are shown in Table 12. It is noteworthy that in practically every case the quantity of pantothenic acid present in litters weighing approximately 42 gm. or above exceeded the vitamin present in the maternal carcass and liver.

An interesting comparison of the concentration of this factor in new tissue with that of older animals is shown in Table 13. It will be observed that there is a marked difference in the amount of pantothenic acid present per unit of tissue.

Table 12. Bound and Free Pantothenic Acid in Tissues of Rats Postpartum (Stock Ration).

Rat Number	Age in Days	Carcass		Liver			Young		
		Per Gm.	Total	Per Gm.	Total	Free	Per Gm.	Total	Free
		mcg.	mcg.	mcg.	mcg.	% of Total	mcg.	mcg.	% of Total
49202	90	5.9	688	94	734	1.1	41.4	1807	89
49232	93	5.5	654	76	718	3.7	38.5	2158	80
49239	92	-	-	94	802	3.2	44.1	1445	79
50259	108	9.3	1059	76	625	2.4	39.8	1663	83
50900	102	7.6	1033	104	842	-	44.1	2252	-
50915	98	7.8	1016	96	794	-	40.2	2065	-
50946	88	8.4	1119	94	722	-	55.8	1188	-
50947	87	7.9	1044	94	830	-	46.2	2233	-
50948	89	7.4	963	94	901	-	54.9	1892	-
Average		7.5	947	91	774	2.6	44.3	1856	83

While practically all of the pantothenic acid present in maternal livers was in the combined form, the same was not true in tissues of newborn rats. See Tables 12 and 14. Assays of young rat tissue, immediately after birth, revealed that approximately 83 per cent of the vitamin was present in the

Table 13. A Comparison of the Concentration of Pantothenic Acid in Animals of Different Ages.

Number of Animals	Age	Pantothenic Acid Mcg. per Gm.
9 litters	Newborn	45
4	6 weeks	18
4	10 weeks	15
8	13 weeks (not mated)	12
9	13 weeks (postpartum)	12

free form. These results were so striking that additional studies were conducted to confirm the observation. Proof that this amount was not due to release of bound pantothenic acid by autolysis was obtained when a litter of newborn rats taken from the stock colony was sacrificed. Tissue homogenate was prepared quickly and boiled for 8 minutes in order to completely inactivate autolytic enzymes. The concentration of free pantothenic acid after such treatment was found to be 30.3 mcg. per gm. of tissue. Novelli, Kaplan and Lipmann have reported that free pantothenic acid represented only a

Table 14. Bound and Free Pantothenic Acid in Tissues of Rats Postpartum (Ration Supplemented with Calcium Pantothenate).

Rat Number	Age in Days	Carcass		Liver			Young		
		Per Gm.	Total	Per Gm.	Total	Free	Per Gm.	Total	Free
		mcg.	mcg.	mcg.	mcg.	% of Total	mcg.	mcg.	% of Total
.002% Calcium Pantothenate on the 16th day									
50297	98	7.0	944	90	850	1.1	62	3506	57
50298	96	8.2	1101	84	658	1.8	80	2368	50
.005% Calcium Pantothenate on the 16th day									
50892	98	8.6	1101	99	755	-	69	3029	-
50894	96	7.5	1066	103	854	1.7	87	3645	54
50914	92	9.5	1132	103	825	-	70	3279	-
50917	94	10.6	1393	99	921	-	71	3017	-
.005% Calcium Pantothenate on the 1st day									
50891	96	9.7	1291	73	545	2.1	67	2870	72
50902	93	9.5	1264	83	719	2.7	69	3877	67

small fraction of the total vitamin present in most tissues, an exception being found in rabbit skeletal muscle which contained equal amounts of the free and bound forms.

Some speculations might be made about the high pantothenic acid content of newborn tissues and concerning the unusual distribution of free and combined forms of this vitamin in young rats at birth.

Possibly there is merely an accumulation of this vitamin in the fetus resulting from an overabundance of the factor in the maternal body. It is well known that excessive amounts of other nutrients, i.e., copper, iron, or iodine can be transferred to the developing tissue without having an immediate beneficial effect upon the young. These substances may be stored for future use. The investigations of Unna (1940) and Hegsted and Perry (1948) have demonstrated rapid depletion of young rats and day-old ducklings unless new sources of pantothenic acid are supplied. Twenty-one day old rats showed deficiency symptoms within 2 weeks when a pantothenic acid deficient ration was fed. Ducklings failed to grow after 2 or 3 days and died within 7 days unless the ration contained pantothenic acid. These observations would seem evidence that the vitamin was not merely accumulating for unimportant stores.

The sharp decline in urinary output of the vitamin by the adult rat just preceding parturition also seems to be

further reason to believe that the amount of vitamin being deposited was physiologically sound.

The very large portion of total pantothenic acid present in the free form in newborn rats suggests that possibly there is another role of this vitamin beyond that of being an essential constituent of coenzyme A. Before pantothenic acid had been shown to be a vital component of this enzyme system with a function in carbohydrate metabolism, Unna and Richards (1942) suggested that this vitamin might be connected with metabolic processes involved in the formation of new tissue. The function of coenzyme A in carbohydrate metabolism seems inadequate to account for the large requirement for pantothenic acid in young animals. Possibly this function is carried out by the free vitamin.

Although animals ingesting the stock ration have been assumed to be well nourished, concentrations of pantothenic acid in livers and carcasses were increased slightly when the ration was supplemented with the vitamin. As shown in Table 14, carcass and liver tissues from animals receiving the ration supplemented with .005 per cent calcium pantothenate contained as much of the vitamin as animals sacrificed at 10 weeks. The effects of added calcium pantothenate were observed when the ration was supplemented on the 16th day of pregnancy as well as for the entire gestation period.

Both free and total pantothenic acid in young rats were markedly increased by addition of calcium pantothenate to the ration of the mother. Total pantothenic acid was elevated to a greater extent than the free form. The direct relationship between the pantothenic acid content of the ration fed the adult rat and the concentration in young tissue resembles the findings of Gillis, Heuser and Norris (1947). The Cornell workers found that the pantothenic acid content of eggs increased directly with the amount of pantothenic acid of the hen's ration.

Whether or not this higher concentration of the vitamin is beneficial to young rats is not known as no attempts have been made to judge the viability of the animals, their response to withdrawal of the vitamin, or to periods of stress.

It was interesting also that as more pantothenic acid was transferred to the young that the stores of bound pantothenic acid increased. This phase of the study needs additional investigation.

The deposition of greater amounts of pantothenic acid in tissues of young rats offers an explanation for the decline in urinary excretion of the vitamin immediately preceding parturition observed in females given supplements of pantothenic acid on the 1st or 16th days of pregnancy.

Influence of a Pantothenic Acid Deficiency Induced by Omega-Methylpantothenic Acid on Reproduction in Rats

Since a disorder known as toxemia of pregnancy was first observed in rats fed a ration containing autoclaved pork and dried yeast as the main sources of protein and the B-vitamins, considerable effort has been spent in trying to determine its cause. Of recent years the incidence of this syndrome has been low and other manifestations of unsatisfactory reproduction have varied. This variability and infrequent development of the disorder has delayed progress in solving the nature of the disease. Recent studies have shown that the experimental diet provided questionable levels of several B-vitamins: riboflavin, pantothenic acid and biotin. Since the females were depleted in pantothenic acid, judging by their liver stores, it was visualized that the transfer of pantothenic acid to the developing young became inadequate, that the young died, and that the depleted females were not able to dispose of the resorbing fetal mass.

There would be several advantages to producing the syndrome on a ration deficient in only one nutrient. Also a diet permitting a higher and more dependable incidence of the disorder would accelerate the solution of the problem. Omega-methylpantothenic acid was therefore incorporated into the customary stock ration so that the effects of a pantothenic acid deficiency might be observed.

Two levels of the analog were first tested, .15 and .30 per cent. Of 8 animals receiving the stock ration plus the analog from the beginning of pregnancy, all animals except one completely resorbed their litters in a rather early stage of the gestation period. These data are given in Table 15.

Table 15. Reproductive Performance of Rats fed the Stock Ration Supplemented with .15 and .30 Per Cent Omega-Methylpantothenic Acid During Pregnancy.

Rat Number	Analog	Number of Young	Number of Resorptions	Wt. Litter	Wt. per rat
	per cent			gm.	gm.
49187	.15	0	4	-	-
49201	.15	0	6	-	-
49231	.15	0	5	-	-
49242	.15	3	7	7.8	2.6
49189	.30	0	3	-	-
49204	.30	0	14	-	-
49234	.30	0	10	-	-
49241	.30	0	12	-	-

Rat # 49242 had been receiving the .15 per cent level of the analog. When this female was killed 22 days after mating it was found that 3 fetuses were alive, one young was dead and partly resorbed, and six others were in various stages of resorption. No gross symptoms of pantothenic acid deficiency were noticed in any of the rats receiving the analog although two females which received the higher amounts

of analog were unusually thin and had rough coats. Upon autopsy it was observed that the livers appeared normal but that in certain cases the adrenals were enlarged. Hemorrhagic adrenals were found in two animals of the group. Judging from the weight patterns of the animal, resorptions had occurred early in pregnancy.

Since "toxemia" encountered in the animals fed the pork diet always occurred during the latter portion of pregnancy, an attempt was made to create an acute deficiency while fetal tissue was developing most rapidly. The ration supplemented with .15 per cent pantothenic acid analog was therefore fed to three females from the 16th day of pregnancy and to one animal from the 18th day. Table 16 shows that reproduction was essentially normal in these animals. Although there were some resorptions, the incidence was no greater than among rats given extra pantothenic acid. The fact that the females continued to gain weight throughout pregnancy would indicate that the resorptions occurred early, before the amount of fetal tissue was large and before the ration was supplemented.

A third attempt to precipitate "toxemias" of pregnancy was made by feeding the stock ration containing .05 per cent omega-methylpantothenic acid. Since previous levels had produced early resorptions it was expected that .05 per cent might result in a more borderline deficiency. After two rats had produced living young on this ration, the level of

inhibitor was increased to .075 per cent. Reproductive performances of these animals are shown in Table 17. No rats developed "toxemia" and reproduction appeared normal in all rats except # 50963 and # 50931. The average birth weights of the young rats were slightly lower than in previous groups.

Table 16. Reproductive Performance of Rats fed the Stock Ration Supplemented with .15 per cent Omega-Methylpantothenic Acid during the Latter Part of Pregnancy.

Rat Number	Day Analog Added	Number of Young	Number of Resorptions	Wt. Litter	Average Weight per Rat
				gm.	gm.
50258	16	9	3	40.9	4.5
50260	16	9	3	45.2	5.0
50262	16	11	0	52.3	4.8
50261	18	12	0	56.8	4.7
Average		10.3	1.5	48.8	4.8

Animals # 50916 and # 50963 developed rough coats and incrustations around the mouths during the latter part of pregnancy. Parturition was extremely slow for animals # 50963 and # 50931. In both cases delivery extended over 3 hours. After the animals were killed, autopsies revealed that live fetuses were still in the uteri. Rat # 50931 showed 12 implantation sites and only four young, including

the one removed from the uterus. Animal # 50963 gave birth to three rats having no visible defects, and three live fetuses were removed from the uterus. Two fetuses were in the process of resorption and no tissue remained on the other three sites of implantation.

Table 17. Reproductive Performance of Rats fed the Stock Ration Supplemented with .05 and .075 per cent Omega-Methylpantothenic Acid.

Rat Number	Per Cent Analog	Number of Young	Number of Resorptions	Wt. Litter	Average Weight per Rat
				gm.	gm.
50916	.05	10	1	41.4	4.1
50918	.05	10	0	44.0	4.4
50940	.05*	8	0	37.3	4.7
50942	.05**	7	1	32.1	4.6
50963	.05***	6	5	24.3	4.1
50931	.075	4	8	15.4	3.9
50960	.075	11	0	44.8	4.1
50962	.075	9	1	38.1	4.2

*Increased to .075 per cent on 7th day.
**Increased to .075 per cent on 5th day.
***Increased to .075 per cent on 3rd day.

To prove that the abnormalities observed with the feeding of omega-methylpantothenic acid resulted from pantothenic acid deficiency and not toxicity of the analog, rations were formulated containing both analog and calcium pantothenate. The potency of the inhibitor was not known and the first attempt

to reverse its effect was unsuccessful. Addition of .002 per cent calcium pantothenate to a ration containing .15 per cent analog was not sufficient to allow normal reproduction in two rats. When extensive bleeding from the vagina occurred in one animal on the tenth day of pregnancy the amount of calcium pantothenate was increased to .005 per cent. As shown in Table 18 over half of the implantations resulted in resorptions in the two animals fed the lower supplements of calcium pantothenate.

Table 18. Reproductive Performance of Rats fed the Stock Ration Supplemented with .15 per cent Omega-Methylpantothenic Acid plus .002 per cent and .01 per cent Calcium Pantothenate.

Rat Number	Per Cent Calcium Pantothenate	Number of Young	Number of Resorptions	Wt. Litter	Average Weight per Rat
				gm.	gm.
50272	.002*	6	5	27.5	4.6
50273	.002	5	7	20.0	4.0
50941	.01	11	0	51.0	4.6
50958	.01	9	0	40.1	4.5
50959	.01	10	1	48.2	4.8
50961	.01	6	4	35.1	5.9

*Calcium pantothenate increased to .005 per cent on 10th day.

When the level of calcium pantothenate was increased to .01 per cent, reproduction appeared to be normal in three animals while there were four resorptions and ten implantation sites in the fourth.

While the main objective in feeding the pantothenic acid analog was not achieved, it appears that this technique for precipitating "toxemia" in pregnant animals should not be abandoned before a level of .10 per cent omega-methylpantothenic acid has been tested. Possibly also there might be value in including a larger quantity of the inhibitor during the middle or end of pregnancy. These experiments are anticipated for future work.

The results of the present section of work confirm observations of others regarding the importance of pantothenic acid during reproduction in the rat.

Estimates of the pantothenic acid activity of the tissues and urine of rats fed the stock ration including omega-methylpantothenic acid were attempted although it is recognized that the values represent only relative changes in the concentration of the vitamin. Drell and Dunn (1948) have shown that the antibacterial index of omega-methylpantothenic acid (the molar ratio of inhibitor to growth promoter which inhibits completely the growth of the organism) differs for the two assay organisms used. Lactobacillus casei which was used in the assay of urine specimens of animals receiving .30 and .15 per cent analog throughout pregnancy, was found to be more sensitive to the inhibitor than Lactobacillus arabinosus which was used later. The relationship between inhibition of growth in lactic acid

bacteria and the production of pantothenic acid deficiency in the rat is also not known. A portion of the data will be presented, however, in spite of these uncertainties, since they may prove of value in future work.

Apparent excretions of pantothenic acid by animals fed the stock ration supplemented with .30 and .15 per cent analog throughout pregnancy are shown in Table 19 and Figures 11 and 12. It will be recalled that no young were produced by females receiving the higher quantity of the analog and that one female produced three living young when .15 per cent of the inhibitor was fed.

When .15 per cent omega-methylpantothenic acid was incorporated into the ration on the 16th day of pregnancy, the females were able to deliver their young and the fetal mass was 35 to 51 gm. Excretion values for such animals are given in Table 20 and Figure 13.

When .002 per cent calcium pantothenate was added to the stock ration which contained .15 per cent inhibitor, reproductive performance was superior to that observed for animals getting only the inhibitor. It was not equal to that noted for rats receiving higher amounts of the vitamin or reduced levels of the analog. Typical excretion values for animals receiving both the inhibitor and added calcium pantothenate are given in Table 21 and Figure 14.

Table 19. Apparent Daily Excretion of Pantothenic Acid by Rats Ingesting the Stock Ration Supplemented with Two Levels of Omega-Methylpantothenic Acid.

Rat Number	Day of Pregnancy 4	8	10	12	14	16	18	20	22
.15% Analog									
49187	183	96	88	69	71	110	80	71	71
49201	152	73	57	67	64	70	60	67	75
49231	206	101	79	62	59	73	68	68	63
49242	198	104	87	80	83	78	79	82	91
.30% Analog									
49189	137	103	70	55	44	40	54	52	-
49204	146	96	61	46	40	50	52	51	53
49234	225	72	52	52	58	52	54	54	53
49241	121	78	67	61	54	49	53	50	58

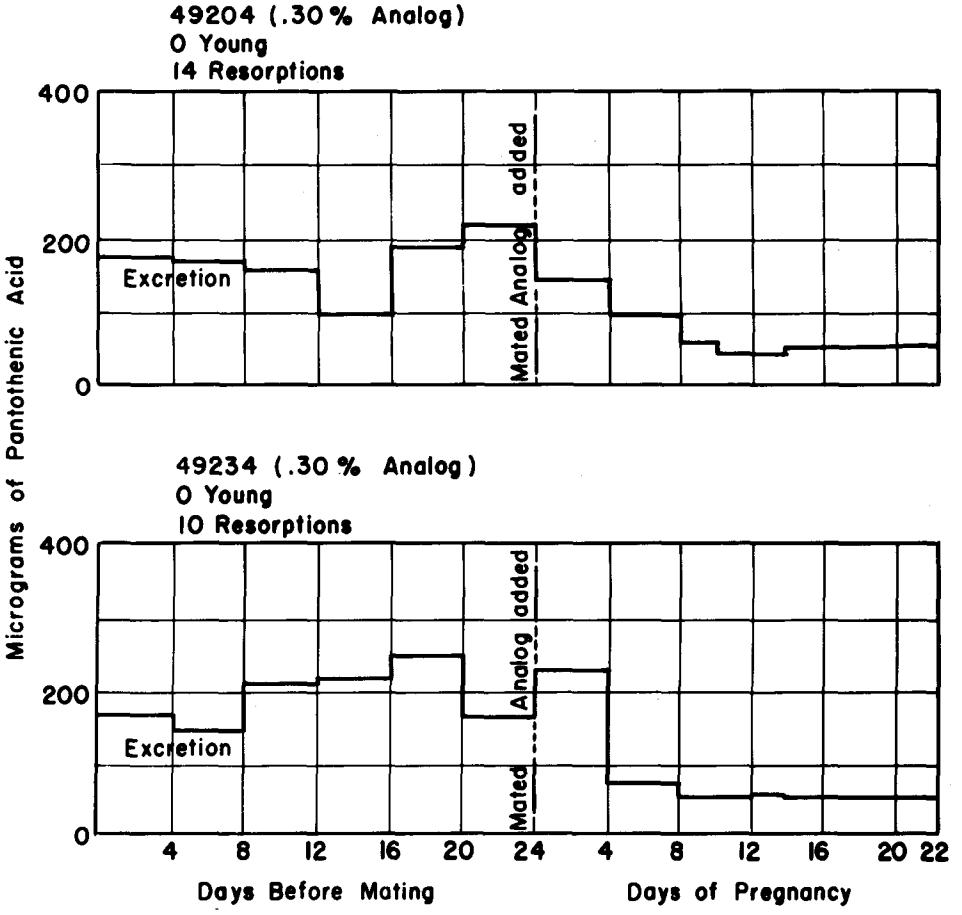

FIGURE 11. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #49204 AND #49234 CONSUMING THE RATION SUPPLEMENTED WITH .30 PER CENT OMEGA-METHYLPANTOTHENIC ACID.

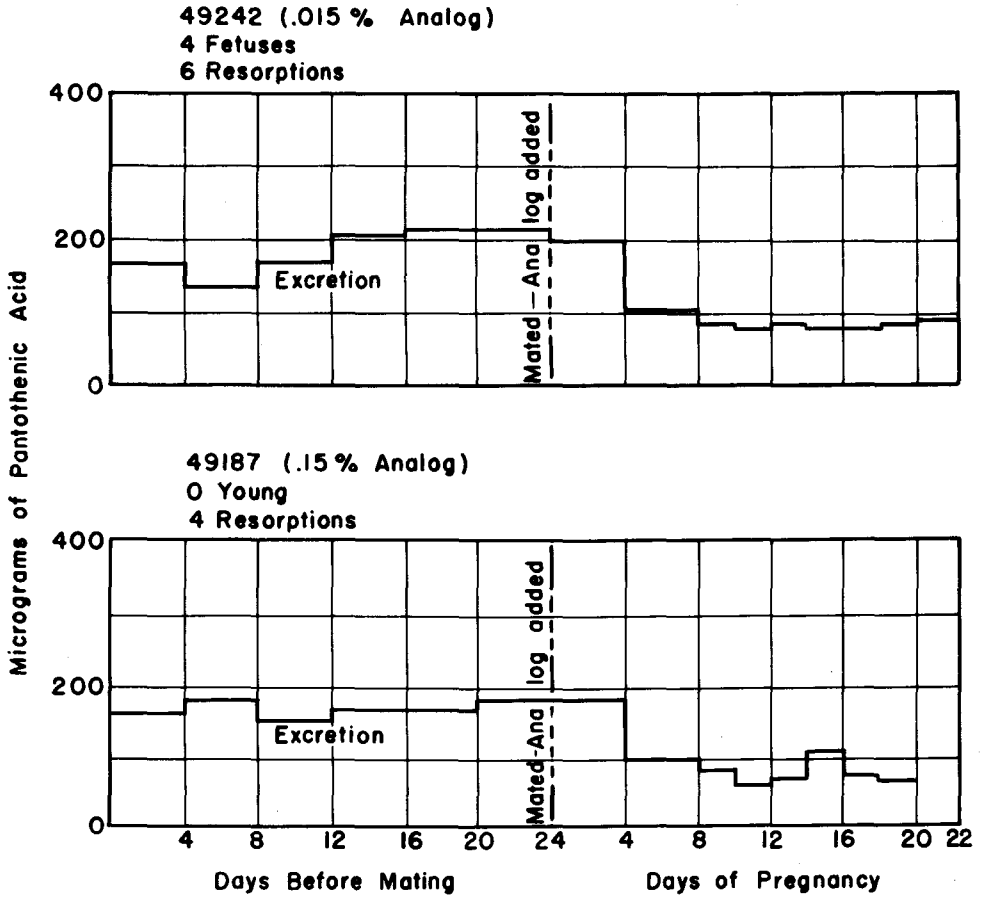

FIGURE 12. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #49187 AND #49242 CONSUMING THE RATION SUPPLEMENTED WITH .15 PER CENT OMEGA-METHYLPANTOTHENIC ACID.

Table 20. Apparent Daily Excretion of Pantothenic Acid by Rats Ingesting the Stock Ration Supplemented with .15 Per Cent Omega-Methylpantothenic Acid on the 16th Day of Pregnancy.

Rat Number	Day of Pregnancy 4	8	10	12	14	16	18	20	22
50258	168	174	204	97	134	175	241	123	73
50260	128	140	166	135	153	113	190	111	96
50262	175	198	172	169	171	157	255	125	67
50261	206	227	180	116	165	220	89	193	109

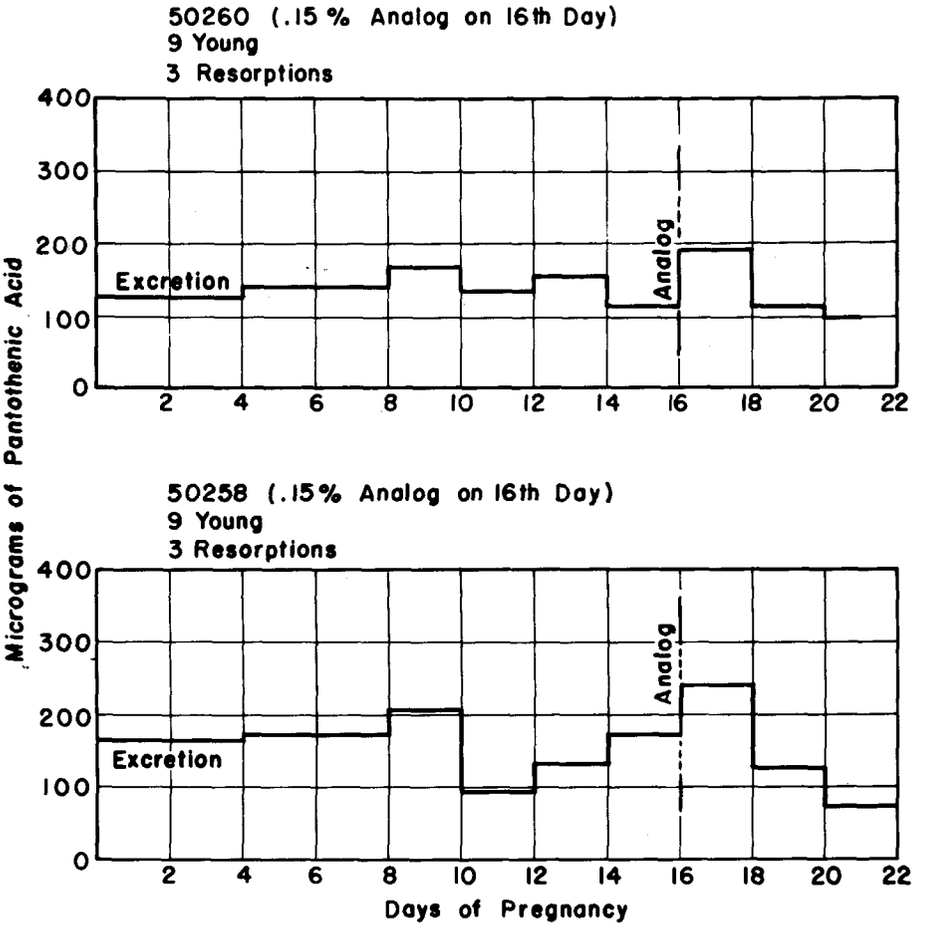

FIGURE 13. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #50258 AND #50260 CONSUMING THE RATION SUPPLEMENTED WITH .15 PER CENT OMEGA-METHYLPANTOTHENIC ACID FROM 16TH DAY.

Table 21. Apparent Daily Excretion of Pantothenic Acid by Rats Ingesting the Stock Ration Supplemented with .15 Per Cent Omega-Methylpantothenic Acid plus .002 Per Cent Calcium Pantothenate.

Number	Day of Pregnancy 4	8	10	12	14	16	18	20	22
50272*	174	101	83	128	142	148	138	155	131
50273	251	154	133	107	140	120	115	109	109

*Intake of calcium pantothenate was increased to .005 per cent on the 10th day.

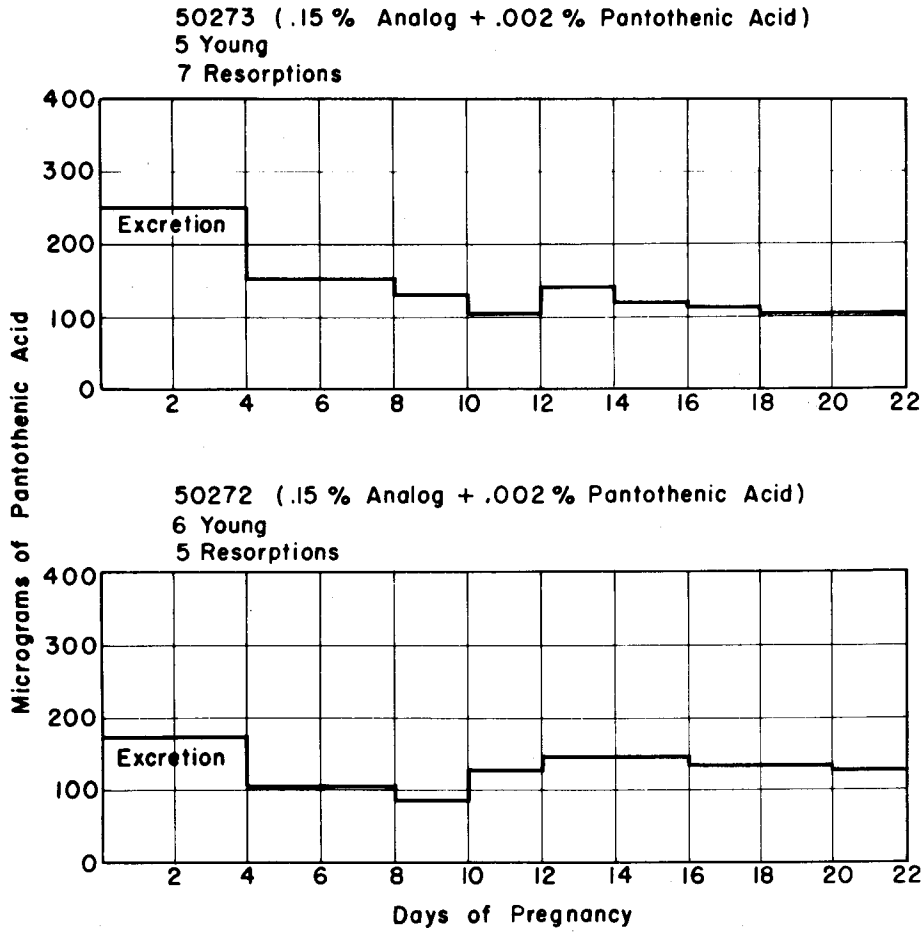

FIGURE 14. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #50272 AND #50273 CONSUMING RATION SUPPLEMENTED WITH .15 PER CENT OMEGA-METHYLPANTOTHENIC ACID PLUS .002 PER CENT PANTOTHENIC ACID.

The true significance of these values is unknown. However it was interesting to observe that when the ration containing the analog was enriched with calcium pantothenate, that the excretion of the vitamin did not fall to the customary low values observed for pregnant animals immediately before parturition. Possibly this apparent excretion of the vitamin was higher because both females were producing small litters.

A summary of the results of pantothenic acid assay of the tissues of females fed the stock ration plus supplements of the analog, or the analog and added calcium pantothenate is given in Table 22. These values are compared with concentrations found in females fed only the stock diet.

It will be observed that as the concentration of vitamin in the tissues became lower, the reproductive performance was poorer. Females which received .30 per cent omega-methylpantothenic acid were unable to produce young. This group of females had unusually low hepatic stores of the vitamin and carcass values were reduced. Decreasing the amount of analog by one-half allowed one animal to bring four young to term. One of these young was dead at parturition. The very low concentration of pantothenic acid in the fetal tissue suggests that the living young might not have survived. Reducing the quantity of analog to .075 per cent permitted more successful reproduction. It will be noted, however, that there was considerable individual variation among the

Table 22. Apparent Concentration of Pantothenic Acid in Tissues of Rats Postpartum fed the Stock Ration Supplemented with Omega-Methylpantothenic Acid.

Rat Number	Supplement	Carcass	Liver	Litter Weight	Fetuses
		mcg. per gm.	mcg. per gm.	gm.	mcg. per gm.
49189	.30% analog	4.6	39	No young produced	
49204		4.2	41	No young produced	
49234		3.5	30	No young produced	
49241		4.5	35	No young produced	
49187	.15% analog	4.3	59	No young produced	
49201		4.3	64	No young produced	
49231		4.5	61	No young produced	
49242		4.0	61	7.8	12.8
50960	.075% analog	4.5	48	44.8	18.7
50942		5.5	38	32.1	20.2
50940		5.6	40	37.3	26.8
50931		4.1	40	15.4	6.9
50963		5.0	43	24.3	27.7
50916	.05% analog	6.3	59	41.4	20.2
50918		5.2	57	44.0	25.5
50258	.15% analog on 16th	4.9	40	40.9	23.9
50260	day of pregnancy	5.6	57	45.2	20.6
50261		5.9	66	56.8	22.8
50262		4.7	58	52.3	19.1

Table 22. (Continued)

Rat Number	Supplement	Carcass	Liver	Litter Weight	Fetuses
		mcg. per gm.	mcg. per gm.	gm.	mcg. per gm.
50272	.15% analog plus	7.0	81	27.5	17.9
50273	.002% Ca Pantothenate	6.1	68	20.0	13.8
50941	.15% analog plus	6.2	65	51.0	28.2
50958	.01% Ca Pantothenate	6.7	83	40.1	28.0
50959		6.1	83	48.2	25.2
50961		6.4	99	35.1	39.9
9 Stock Females		7.4	91	42.3	45.0

females. In this case it was interesting to find that the hepatic stores of the mother seemed to suffer in an effort to continue the development of the young.

When .05 per cent analog was fed, there was somewhat more pantothenic acid in the livers of the mothers; both females produced large litters. The concentration of pantothenic acid per gm. of fetal tissue did not differ greatly from young of females fed .075 per cent analog.

Incorporating omega-methylpantothenic acid into the ration on the 16th day of pregnancy resulted in a lowering of hepatic stores of the female below those of animals fed the stock diet. Likewise the stores of the fetuses were smaller, being more like those of young produced by rats ingesting .05 per cent of the analog.

The addition of .002 per cent calcium pantothenate on the first day of pregnancy aided reproduction. In this case two females carried twelve young to term, although considerable difficulty was observed at parturition and about one-half of each litter had resorbed. The quantity of pantothenic acid present in fetal tissue was small. The addition of .01 per cent calcium pantothenate improved reproductive performance still further. In this case the number of young compared favorably with females receiving the stock ration. Maternal liver stores were more nearly equal to those of the control females. While the concentration of pantothenic

acid in the fetuses was higher than that found in any of the other groups in which the inhibitor was consumed, the average concentration of the vitamin was not equal to that of newborn rats borne to stock animals.

These results have been presented since the apparent stores of pantothenic acid correlated closely with the outcome of pregnancy. If the values reflect the true pantothenic acid activity of the tissues, it would be most valuable to gain additional information concerning the concentration of vitamin in the young which insures survival and normal physiological function. Possibly the desirable quantity is under 45 mcg. per gm. of tissue, that of young produced by stock females. It may be above this amount, more nearly like that of females fed extra pantothenic acid. These problems should be studied more thoroughly.

SUMMARY AND CONCLUSIONS

Quantitative needs for pantothenic acid during gestation in rats have been investigated, as this vitamin appears to be of special importance in reproduction of animals. Interest in the problem in this laboratory evolved from earlier work which suggested a possible relationship between pantothenic acid deficiency and toxemia of pregnancy. The ration which had produced toxemia in pregnant rats close to parturition supplied only 15 per cent as much pantothenic acid as that furnished by the customary stock diet.

In this investigation estimates of the pantothenic acid requirement of the pregnant rat have been based upon changes in the amounts of the vitamin present in tissues and in the excretion of the vitamin by the kidney. The effect of adding calcium pantothenate to the stock ration has been investigated. The influence of a pantothenic acid deficiency upon reproduction has also been studied by means of a competitive analog, omega-methylpantothenic acid, which was incorporated into the stock diet.

Assays of fetal tissues of rats sacrificed at thirteen intervals during the gestation period revealed that 95 per cent of the pantothenic acid present in newborn rats was transferred to the fetuses after the sixteenth day of

pregnancy. The maximum daily increment in total stores of the vitamin in the young was 650 mcg. This maximum need occurred on the 21st day of gestation. The amount of pantothenic acid present in a litter of newborn rats was equivalent to that found in the entire body of the adult female.

These findings indicate that the requirement for pantothenic acid during reproduction is markedly increased, particularly during the latter half of pregnancy and that at least 650 mcg. of the vitamin should be added to the ration satisfactory for maintenance of the non-pregnant adult female.

Analyses of the maternal hepatic and carcass tissues demonstrated that there was no storage or depletion of the vitamin by the adult stock animal which had access to approximately 600 mcg. of pantothenic acid throughout reproduction.

The urinary excretion of pantothenic acid by adult stock rats was equal to approximately one-third of the dietary intake of the vitamin prior to pregnancy or during the first portion of the reproductive cycle. Pregnant rats exhibited practically no change in the amount of vitamin excreted by the kidney until the last 6 days of pregnancy, at which time the surplus vitamin decreased to very small amounts. The fact that such a large percentage of the pantothenic acid present in newborn rats was acquired during these last few

days of pregnancy meant that a greater fraction of the dietary intake of the vitamin was accounted for than was true for the non-pregnant animal. This observation implies that the animal absorbed larger percentages of the dietary vitamin when the need was high.

A question as to the value of the relatively high concentration of pantothenic acid in young rats was raised following the results of supplementing the stock ration with calcium pantothenate. The concentration in the young nearly doubled when 500 mcg. of extra calcium pantothenate was added daily throughout pregnancy. Addition of the salt of pantothenic acid on the sixteenth day of pregnancy or from the first day did not prevent a decline in the urinary excretion of this factor just prior to parturition. If the amount of the vitamin transferred to the developing fetuses reflected the intake of the female rather than an amount necessary for the well-being of the young, the same might have been true when the unsupplemented stock ration was fed. In light of the work of others concerning the rapid depletion of young animals, their inability to survive without added pantothenic acid and their unusual needs for early growth, it is believed that the concentrations present in the young of stock females are desirable. Possibly these amounts do not represent the optimum. Additional studies are needed to investigate this question further.

Determinations of both free and bound pantothenic acid in maternal hepatic tissue and in the newborn revealed that only 2 or 3 per cent of the total pantothenic acid in the maternal liver was present in the free form. In contrast, approximately 80 per cent of the vitamin in the newborn was present as free pantothenic acid.

These unexpected findings, both as to concentration of pantothenic acid in newborn rats and the high proportion of that vitamin present in the free form, allow the speculation that the vitamin may have an important role in the formation of new tissue beyond that suggested for coenzyme A. Is it possible that the portion designated as free pantothenic acid is actually combined in some way which permits utilization by lactic acid bacteria? Is this accumulation merely the result of high dietary intake on the part of the mother? Or does this rapid development of fetal tissue (equal to nearly 40 or 50 gms. in approximately 6 days) mean that synthesis of certain types of tissue requiring pantothenic acid is more rapid at this age than at any other period of life? Additional studies are needed first to determine the correlation between the concentration of pantothenic acid present in newborn rats and their survival, growth or general well-being.

Attempts to develop "toxemia" of pregnancy were unsuccessful at the levels of analog tried. Females fed .15

per cent omega-pantothenic acid from the initiation of pregnancy appeared to develop too severe a vitamin deficiency. Possibly .075 per cent of the inhibitor more nearly approaches the degree of deficiency needed. While the observations suggest that additional use of the analog might precipitate the disorder, more information might be gained in the future through the use of synthetic ration.

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APPENDIX

Table 1. Pantothenic Acid Content of Ration and Supplements

Series of Experiments	Stock Ration	Beef	Carrots
	mcg./gm.	mcg./gm.	mcg./gm.
1	43.9	5.6	5.0
2	56.2	7.4	4.0
3	53.1	5.5	6.0
4	53.7	5.4	5.2

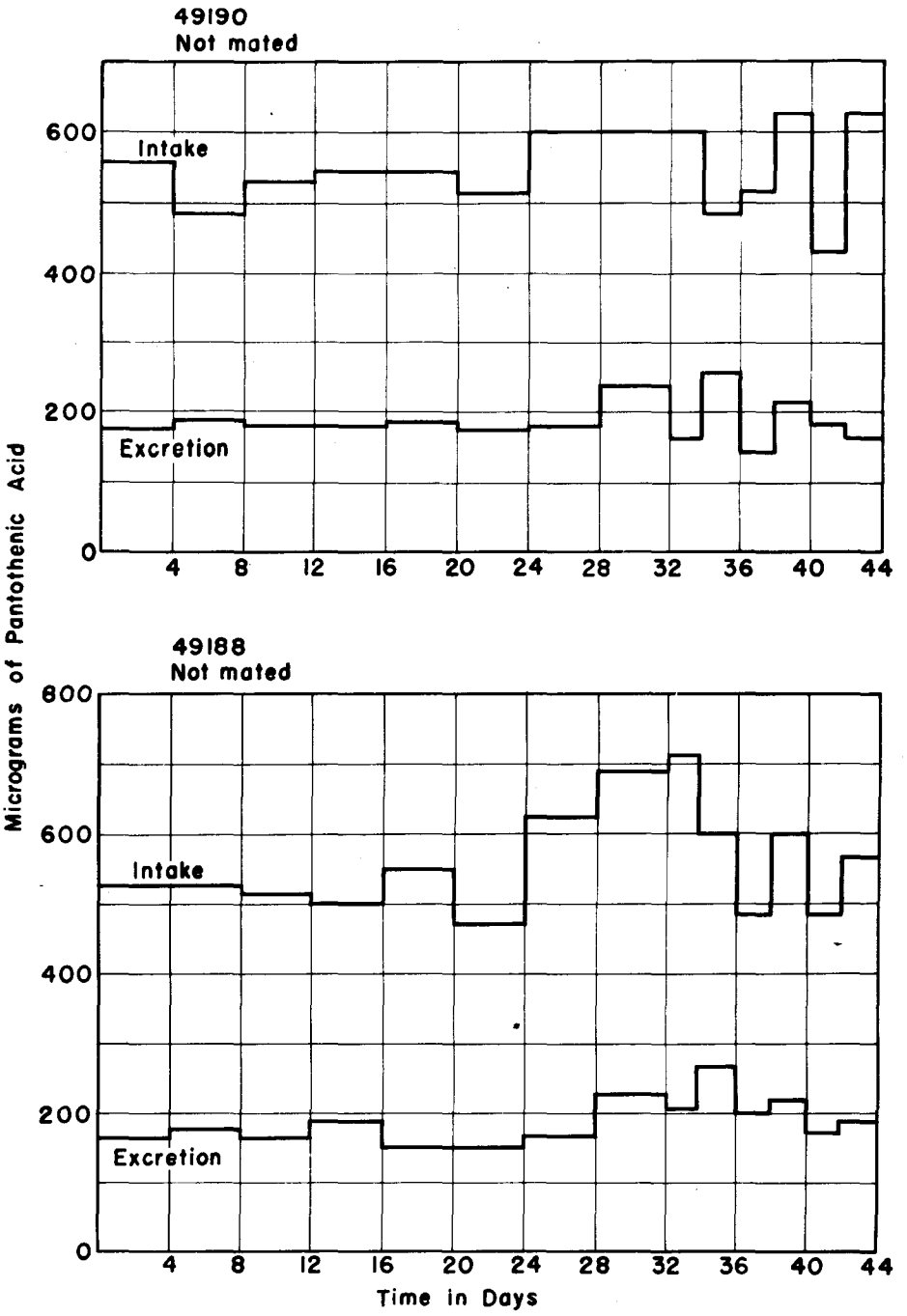

FIGURE 1. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY 6 WEEKS OLD RATS #49188 AND #49190.

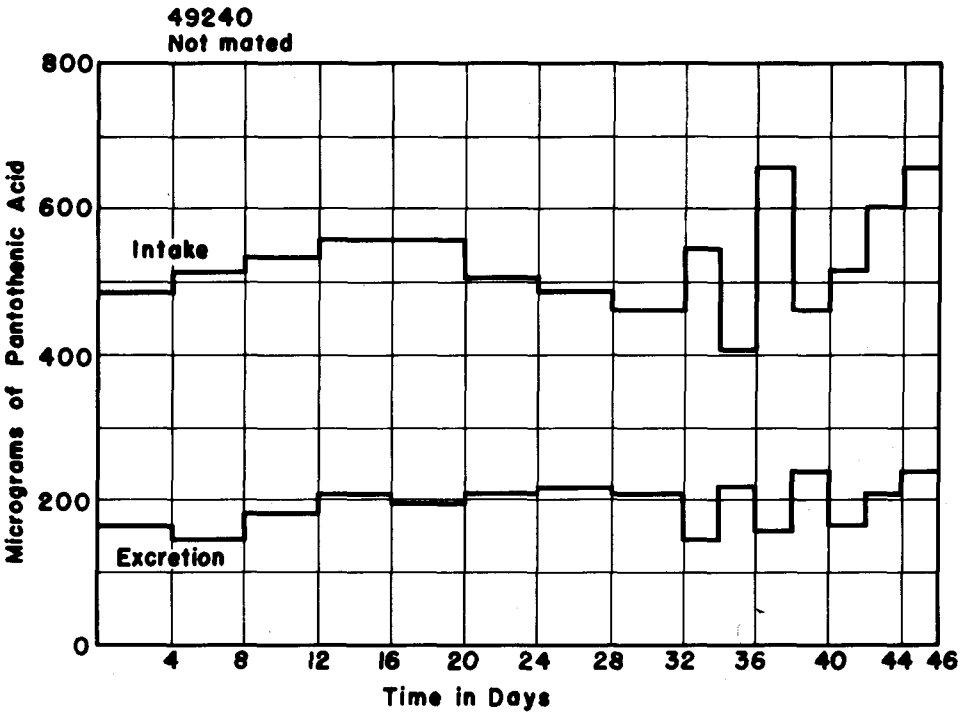

FIGURE 2. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY 6 WEEKS OLD RAT #49240.

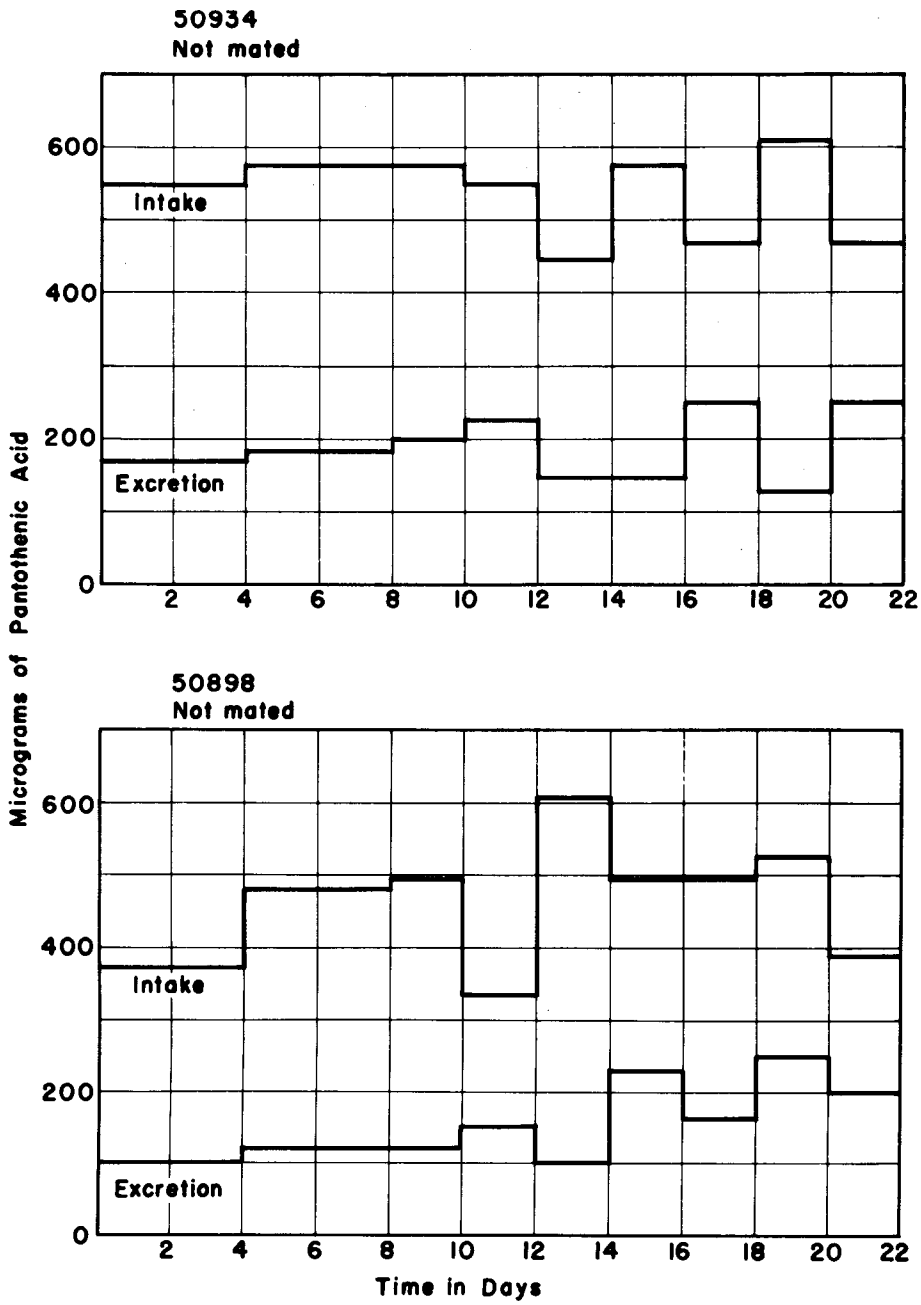

FIGURE 3. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY 10 WEEKS OLD RATS #50898 AND #50934.

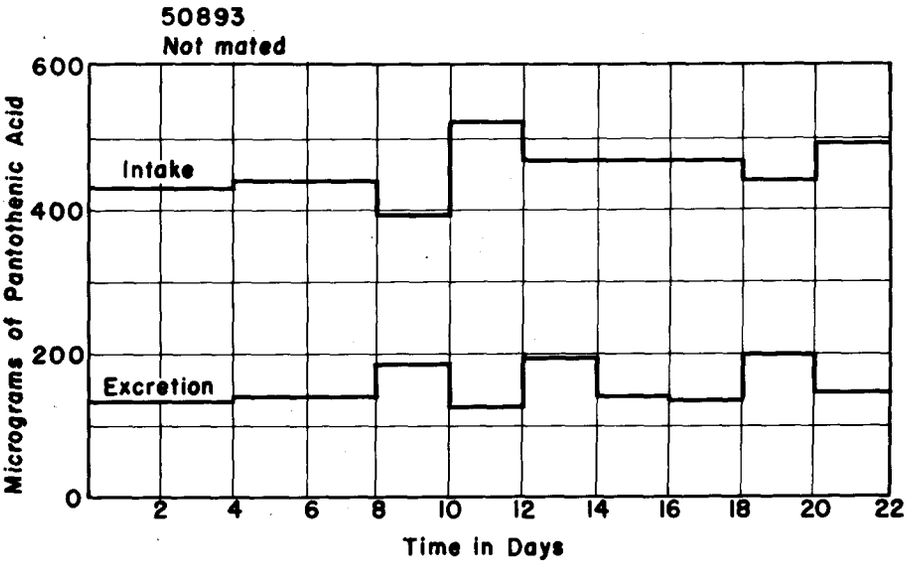

FIGURE 4. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY 10 WEEKS OLD RAT #50893.

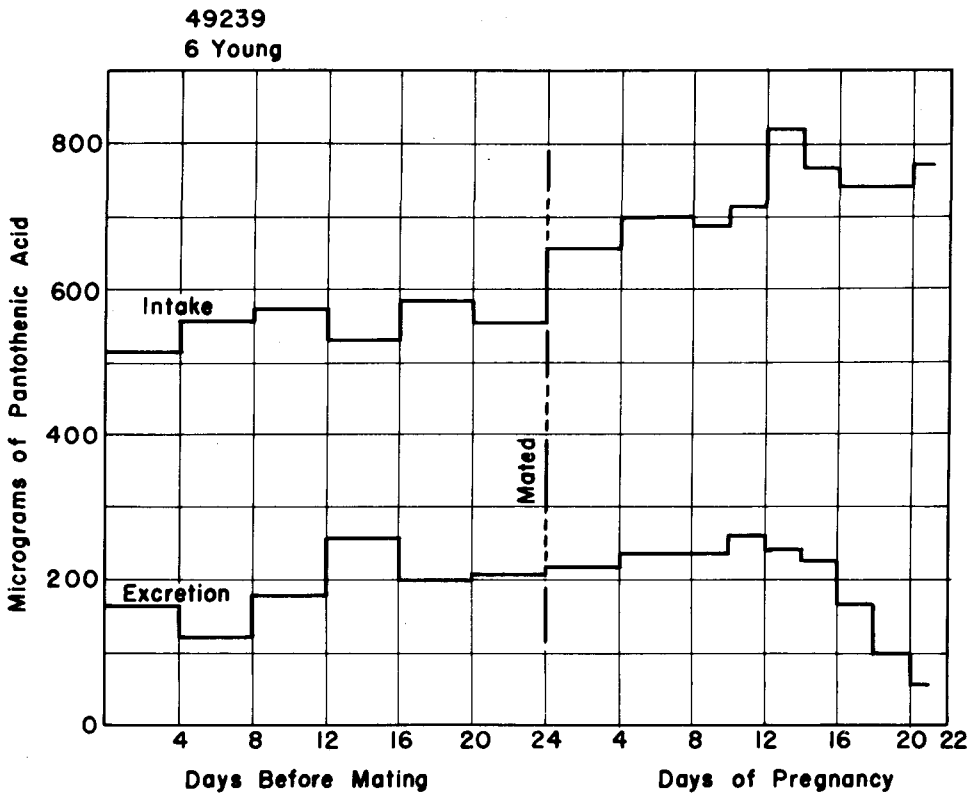

FIGURE 5. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #49239.

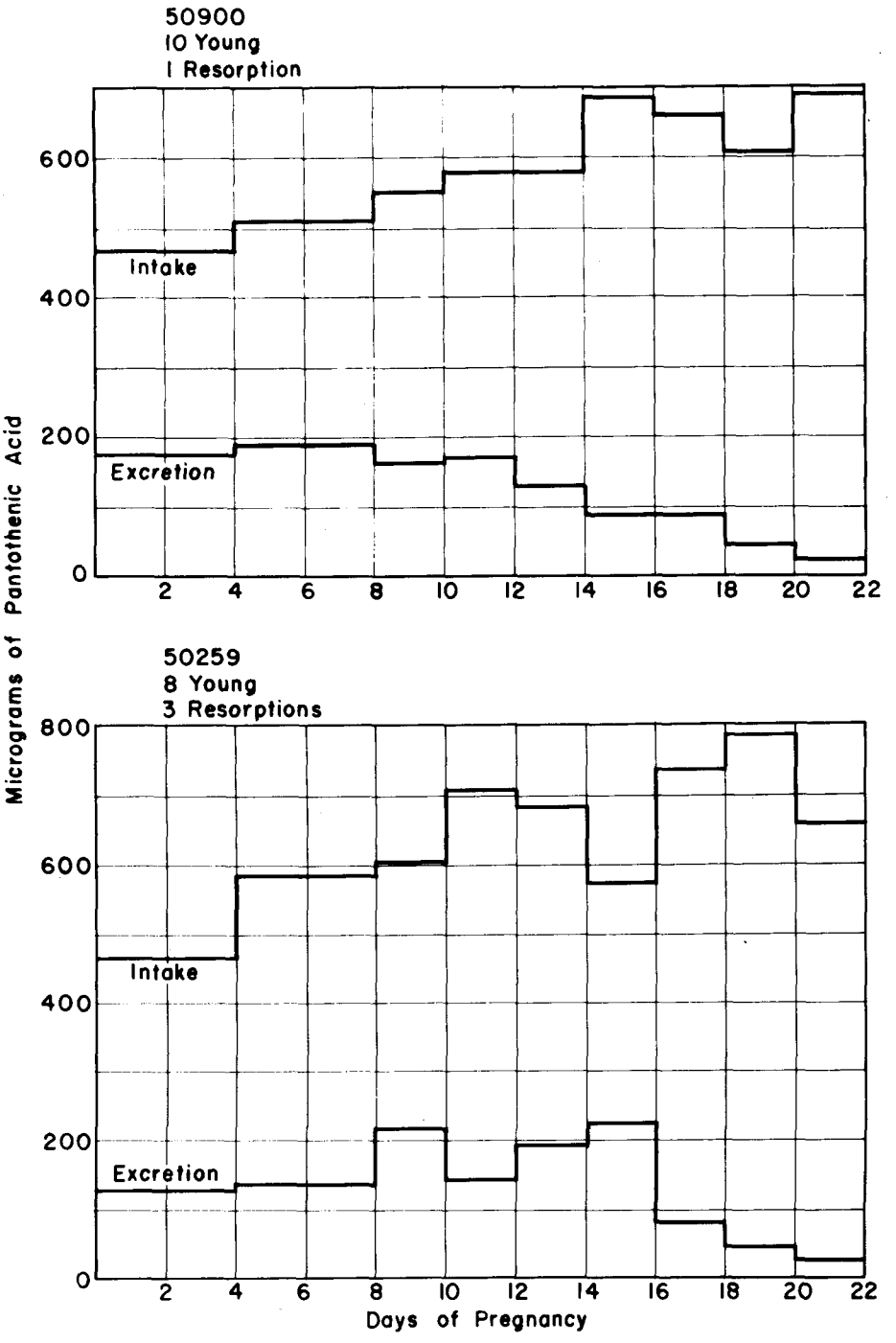

FIGURE 6. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RATS #50259 AND #50900.

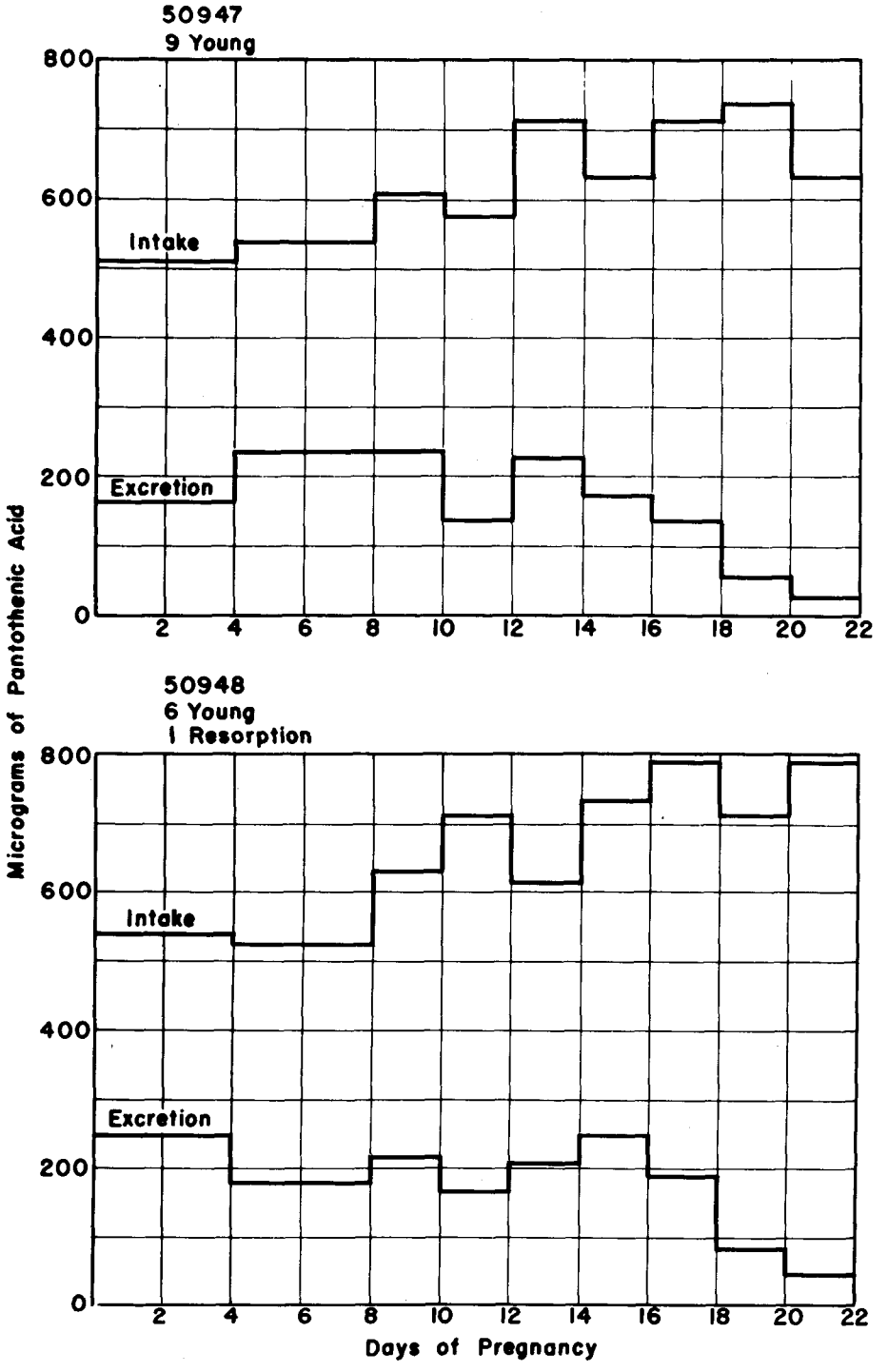

FIGURE 7. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RATS #50948 AND #50947.

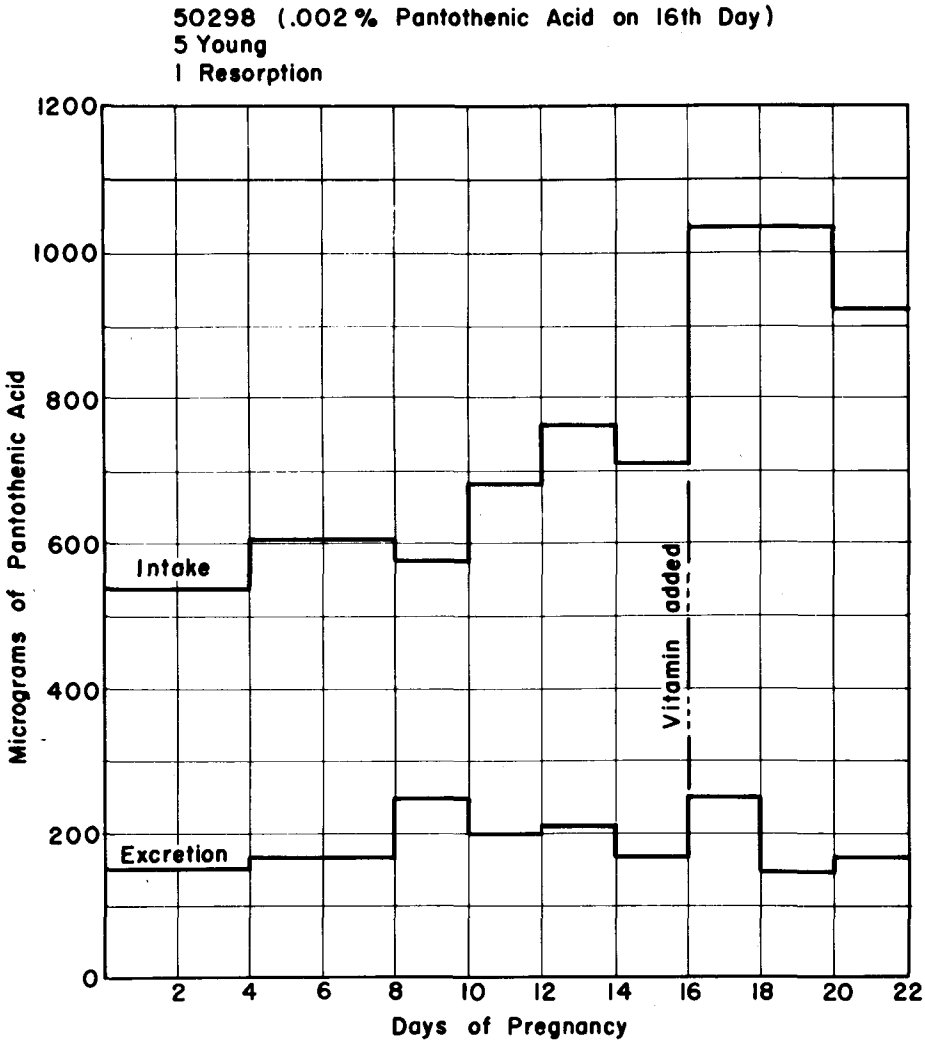

FIGURE 8. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50298 CONSUMING THE RATION SUPPLEMENTED WITH .002 PER CENT PANTOTHENIC ACID ON THE 16TH DAY.

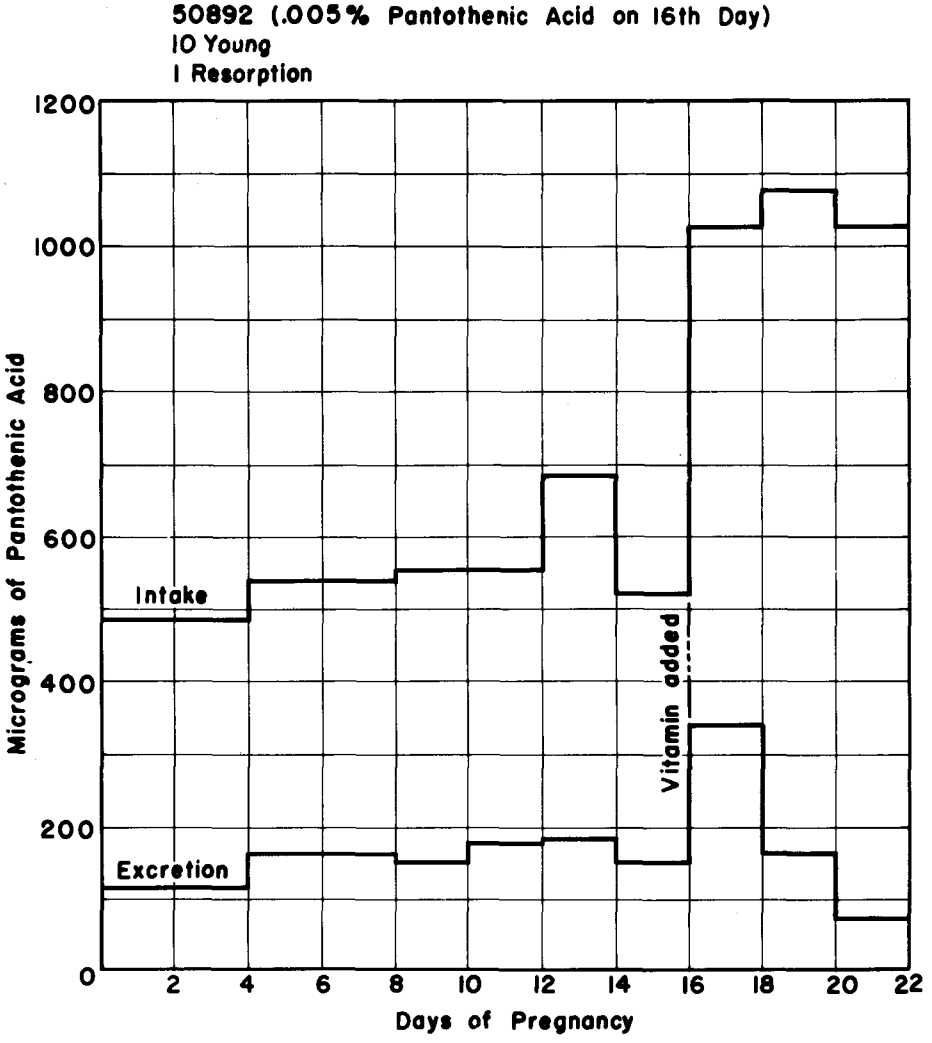

FIGURE 9. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50892 CONSUMING THE RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID ON THE 16TH DAY OF PREGNANCY.

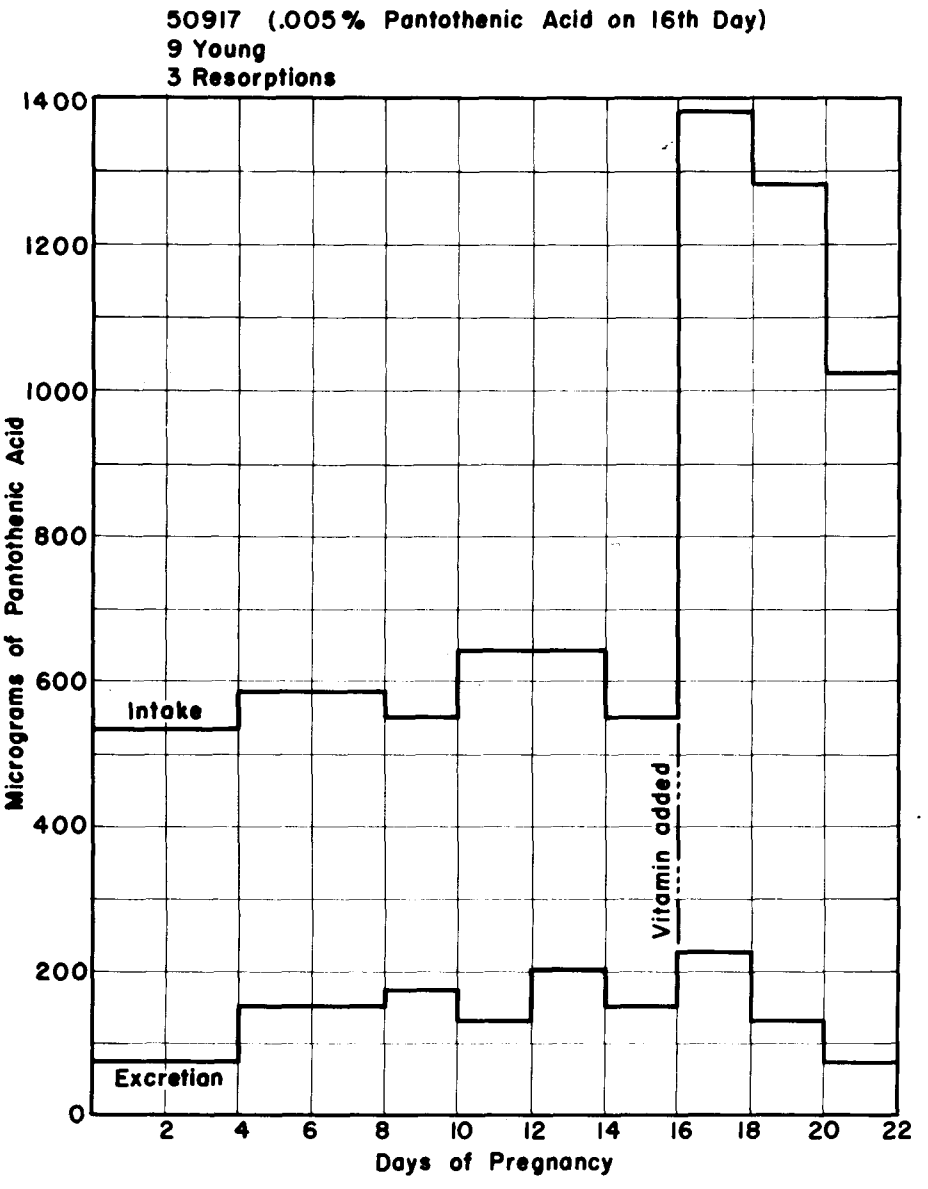

FIGURE 10. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50917 CONSUMING THE RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID ON THE 16TH DAY OF PREGNANCY.

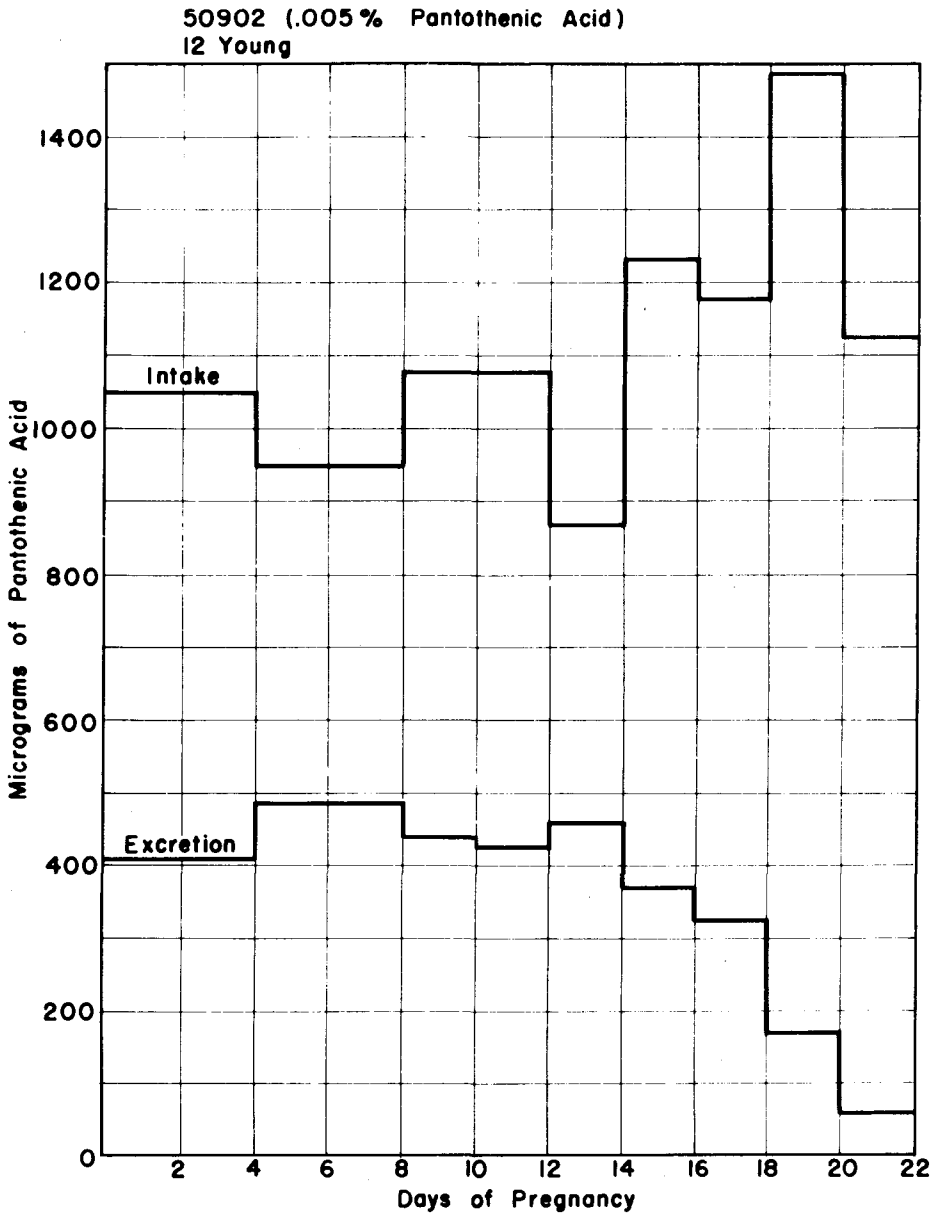

FIGURE 11. INTAKE AND EXCRETION OF PANTOTHENIC ACID BY RAT #50902 CONSUMING THE RATION SUPPLEMENTED WITH .005 PER CENT PANTOTHENIC ACID THROUGHOUT PREGNANCY.

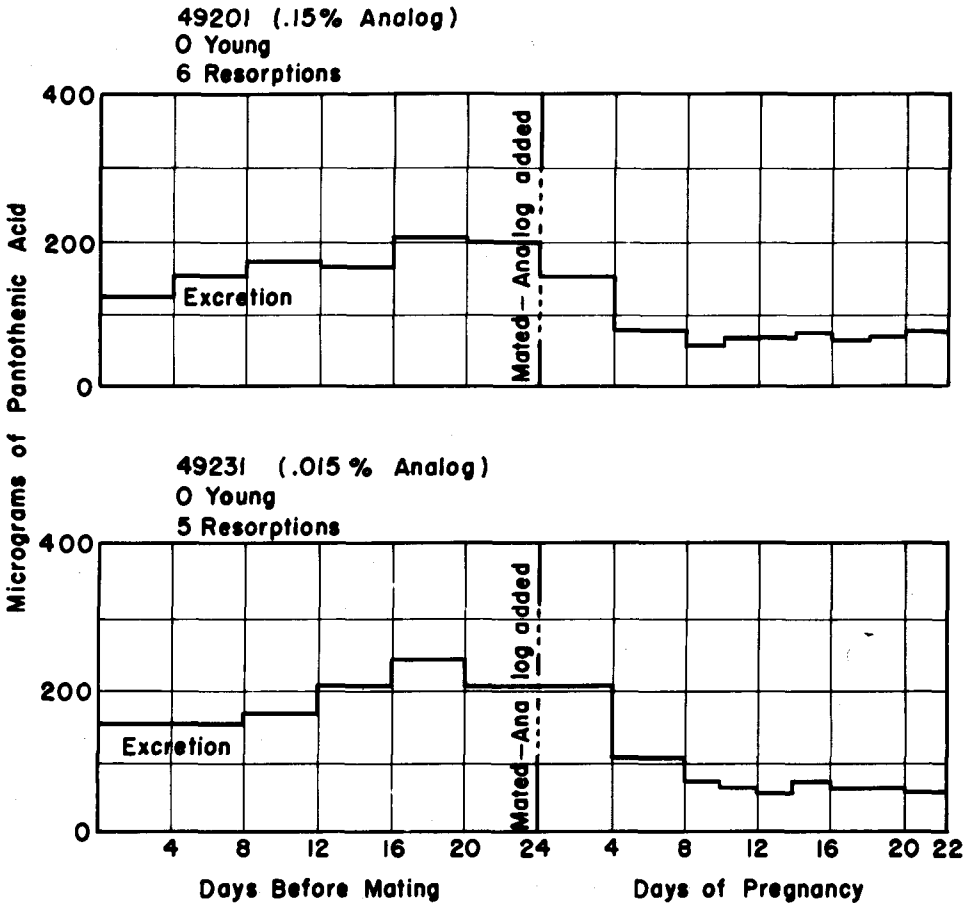

FIGURE 12. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #49231 AND #49201 CONSUMING THE RATION SUPPLEMENTED WITH .015 PER CENT OMEGA-METHYLPANTOTHENIC ACID THROUGHOUT PREGNANCY.

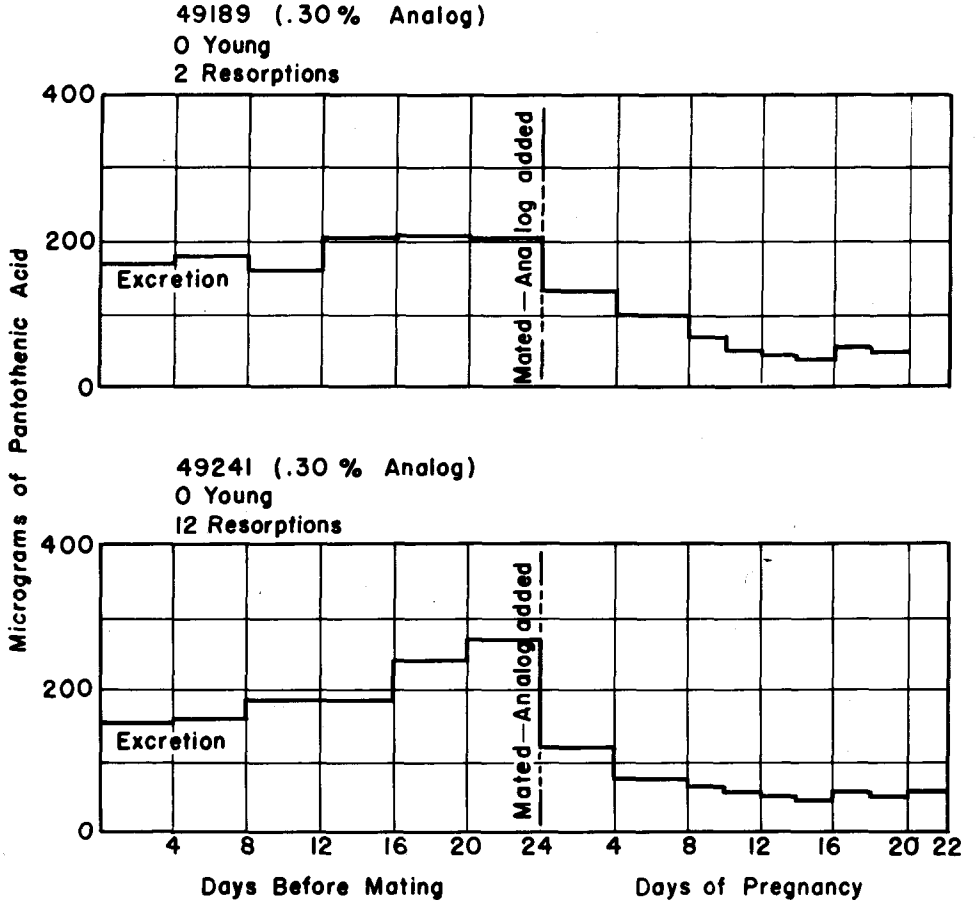

FIGURE 13. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #49241 AND #49189 CONSUMING THE RATION SUPPLEMENTED WITH .30 PER CENT OMEGA-METHYLPANTOTHENIC ACID THROUGH PREGNANCY.

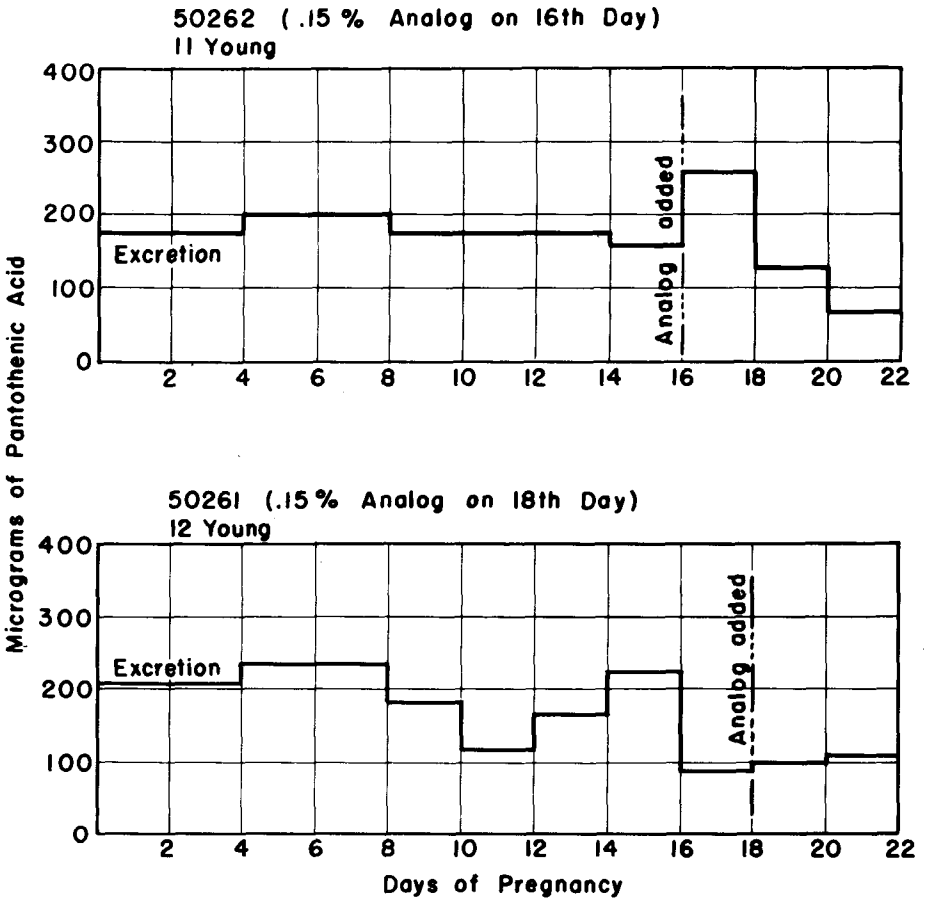

FIGURE 14. APPARENT EXCRETION OF PANTOTHENIC ACID BY RATS #50261 AND #50262 CONSUMING THE RATION SUPPLEMENTED WITH .15 PER CENT OMEGA-METHYLPANTOTHENIC ACID DURING THE LATTER PART OF PREGNANCY.